

Immunology

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Course description

- This is a 3 C.H. course in basic immunology designed for **second-year medical students** to provide them with fundamentals and principles of the human immune system.
- The course will provide the main principles for clinical immune complications

Course outlines

1. Innate and acquired immunity
2. Elements of innate and acquired immunity
3. Immunogens and antigens
4. Antibodies (immunoglobulins) structure and function
5. The biology of B lymphocytes
6. The biology of T lymphocytes
7. Role of major histocompatibility complex in the immune response
8. Activation of T and B cells
9. Cytokines network
10. Complement system
11. Immune tolerance and Autoimmunity
12. Hypersensitivity reactions
13. Tumor immunology
14. Transplantation



Chapter 1: Innate and acquired immunity

Dr. Dalia Wehedi



Learning Objectives:

Upon completion of this lecture you will be able to:

- Define the terms immunity, immunology
- To know the importance of the immune system
- Differentiate innate and adaptive immunity in terms of components, parts and type of immune response.
- To know what are the types of the adaptive system
- To know what are the characteristics of the adaptive immunity

Immunology

- The study of the immune system or immunity
- the study of all aspects of **host defense** against infection and of **adverse consequences** of immune responses.
- The study of the **physiological mechanisms** which enable the body to recognize materials as foreign and to neutralize, metabolize or eliminate them without injury to the host tissue.

➤ Immunity:

- State of protection from infectious diseases

Immunity

- Immunity is body's ability to resist or eliminate potentially harmful foreign materials or abnormal cells

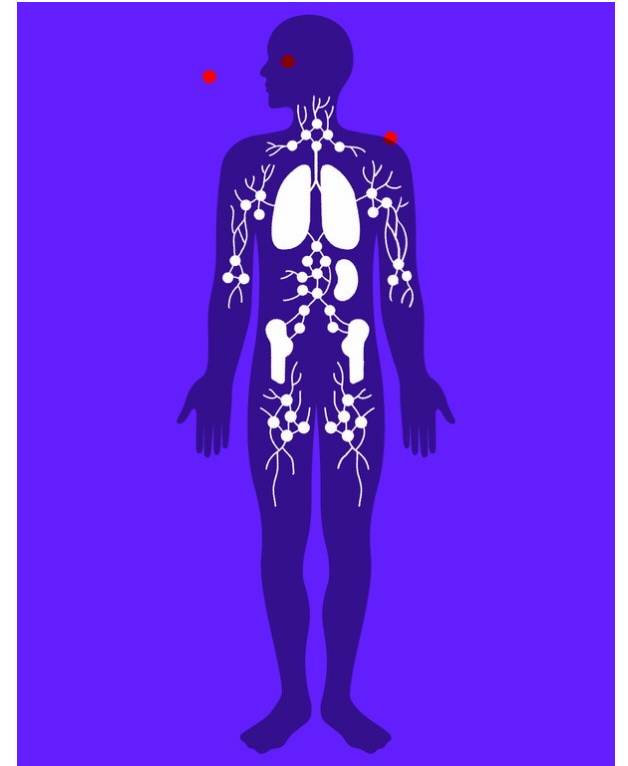


The immune system

A functional system – **NOT** an organ system:

Complex system – includes:

- Skin – physical “mechanical” barrier
- Lining of mucus membranes – a physical barrier
- Secretions – tears, mucus, etc - antimicrobial
- Blood cells and vasculature – WBCs
- Bone marrow
- Liver – makes complement proteins
- Lymphatic system and lymphoid organs
- Most tissues – have resident immune cells “Kupffer cells, dust cells ...”



Immunity

- Immunity (immunis- Latin-exempt, state of protection from infectious diseases)
- Immunity is the body's ability to **resist** or **eliminate** potentially harmful foreign materials or abnormal cells
- Consists of the following activities:
 - Defense against invading pathogens (viruses & bacteria)
 - Removal of 'worn-out' cells (e.g., old RBCs) & tissue debris (e.g., from injury or disease)
 - Identification & destruction of abnormal or mutant cells (primary defense against cancer)
 - Rejection of 'foreign' cells (e.g., organ transplant)
 - **Inappropriate responses:**
 - Allergies - response to normally harmless substances
 - Autoimmune diseases

Why do we need an Immune System?

- Pathogens are microscopic organisms that cause disease
(Each attacks in a specific way)
Viruses, Bacteria, Fungi, Parasites, and Protozoans
- Other environmental substances challenge the lymphatic system
Environmental pathogens (poison ivy, etc)
Abnormal body cells such as cancers
- The Immune system is coupled with the Lymphatic system



Where and how do we defend against disease pathogens?

Many body cells and tissues are involved in the implementation of immunity (Not just lymphocytes and other immune cells)

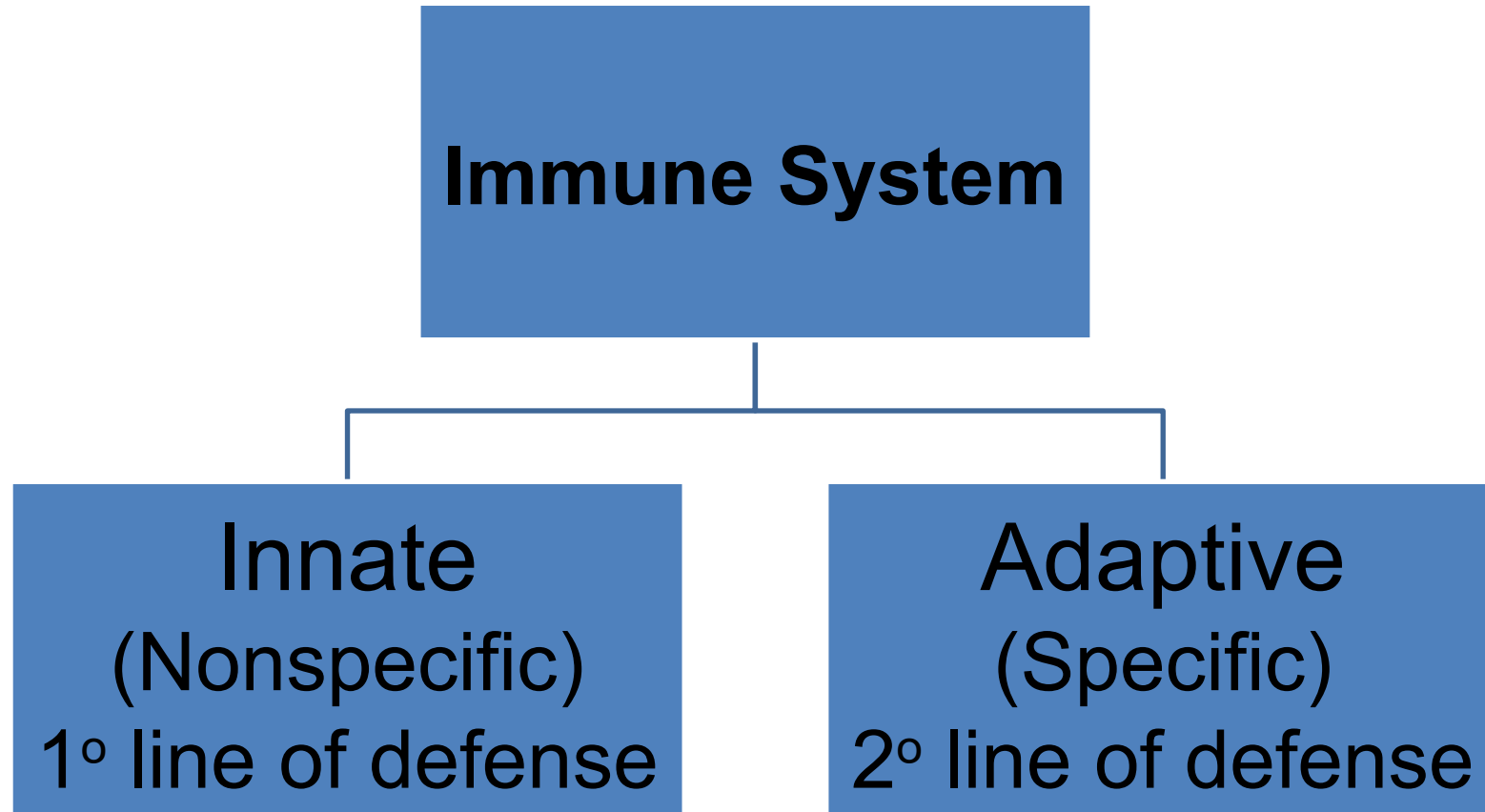
§ Innate (Nonspecific) Defenses (we are born with this capability)

- § Can involve the epithelium on the body surface (integument) or occur in connective tissue, in the GI system and/or may involve a cellular response
- § Respond immediately to many different harmful agents
- § Do not require previous exposure to a foreign substance

§ Adaptive (Specific) Defenses (these components develop with time)

- § Lymphocytes (B, T): Are major players in the immune response but other cells and participants in the innate system work cooperatively “APCs”
- § Identifies, attacks, and reinforces immunity to a specific pathogen

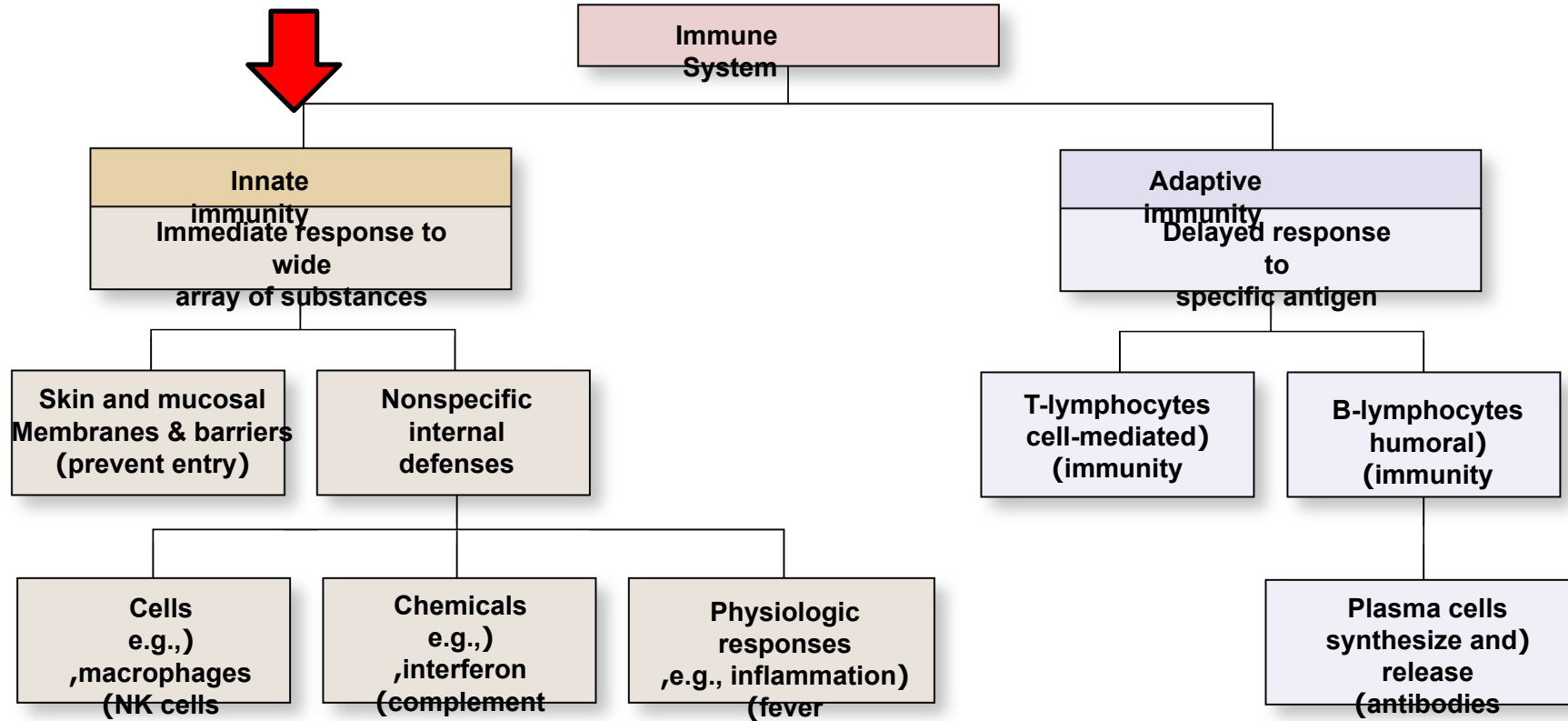
Overview of the Immune System



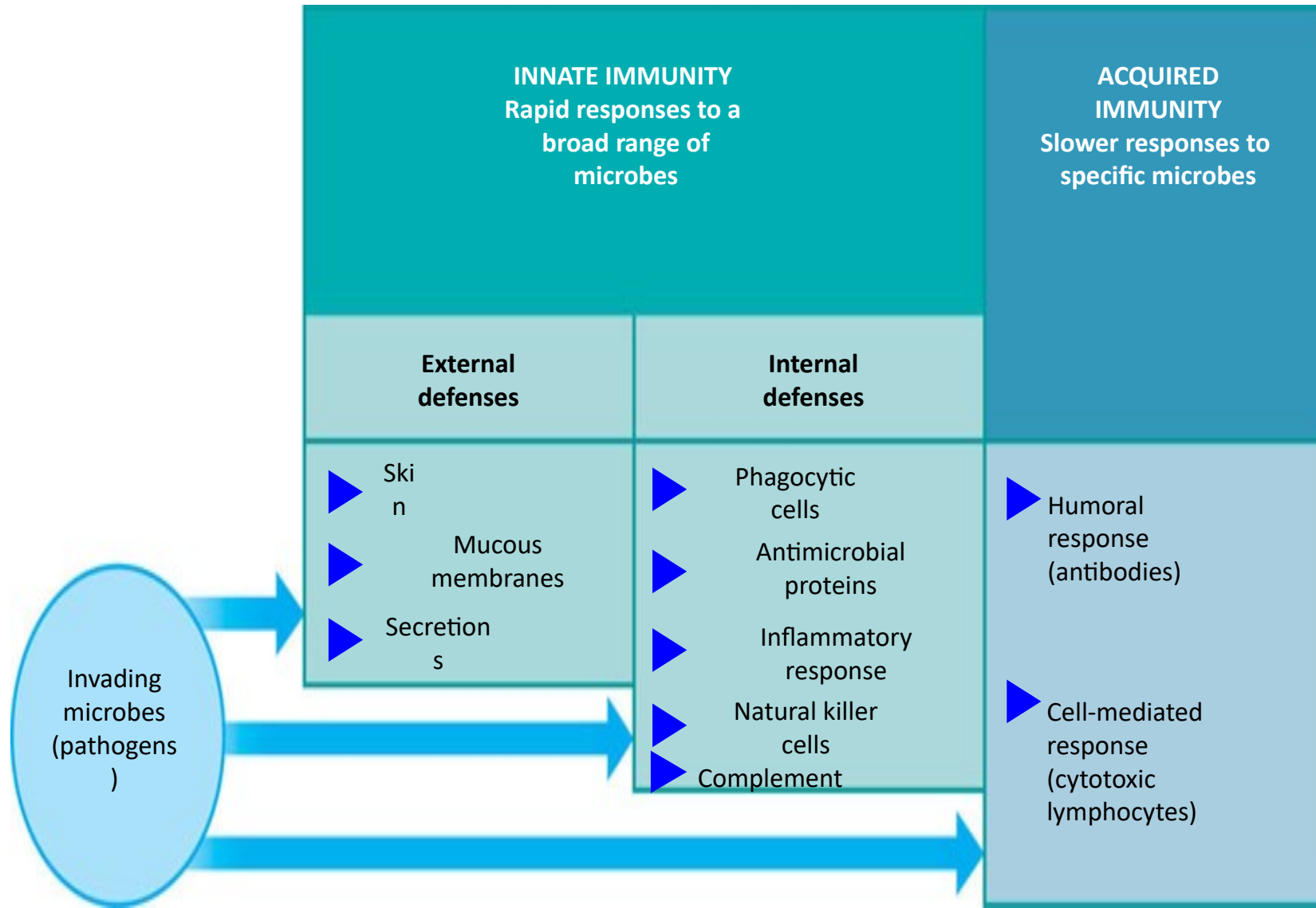
Interactions between the two systems

Overview of the Immune System

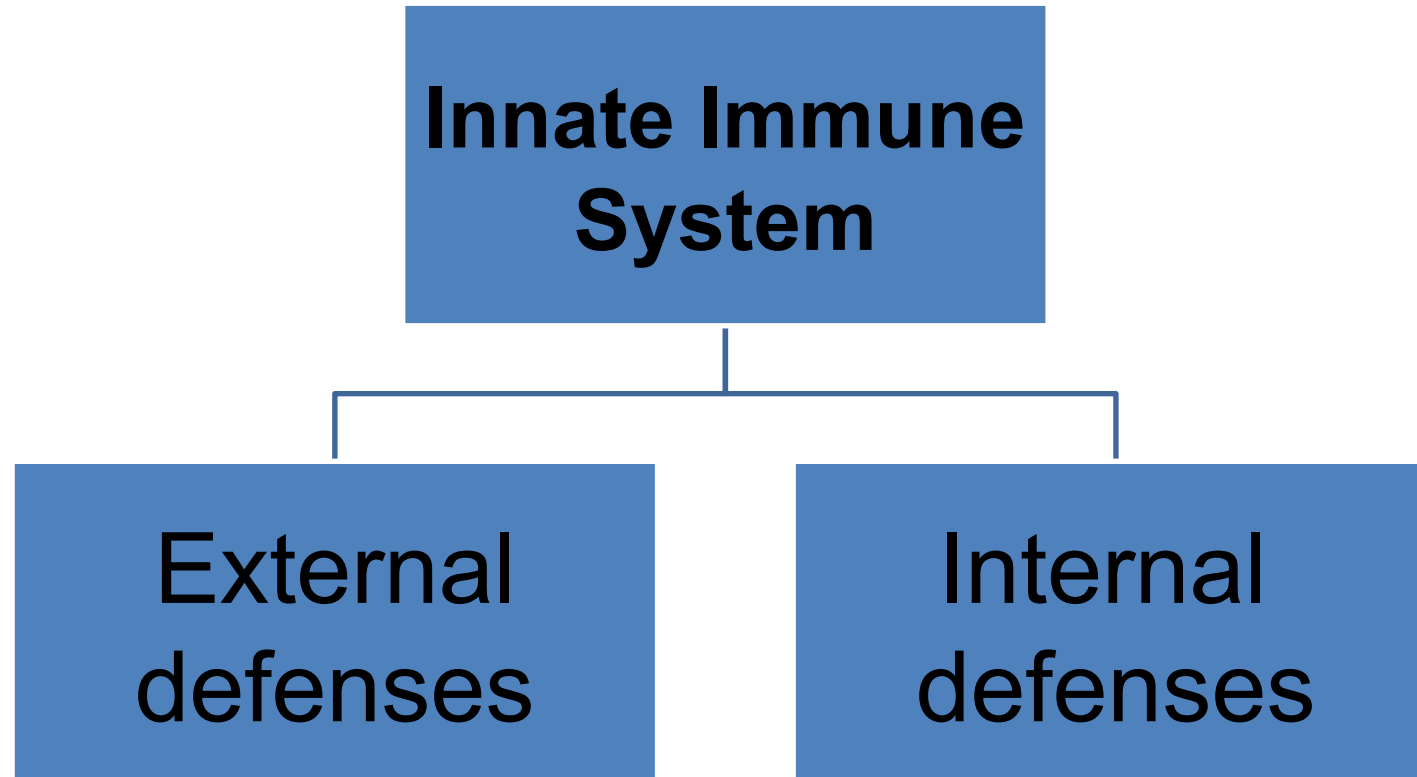
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Typical immune response



The innate immune System



The Innate immunity

Natural immune system (Innate Immunity)

- ✓ Non-specific
- ✓ First line of defense
- ✓ Repeated exposure - no augmentation
- **Components**
 - ✓ Biochemical
 - ✓ Physical
 - ✓ Cells

...cont

1. Components

a. Biochemical

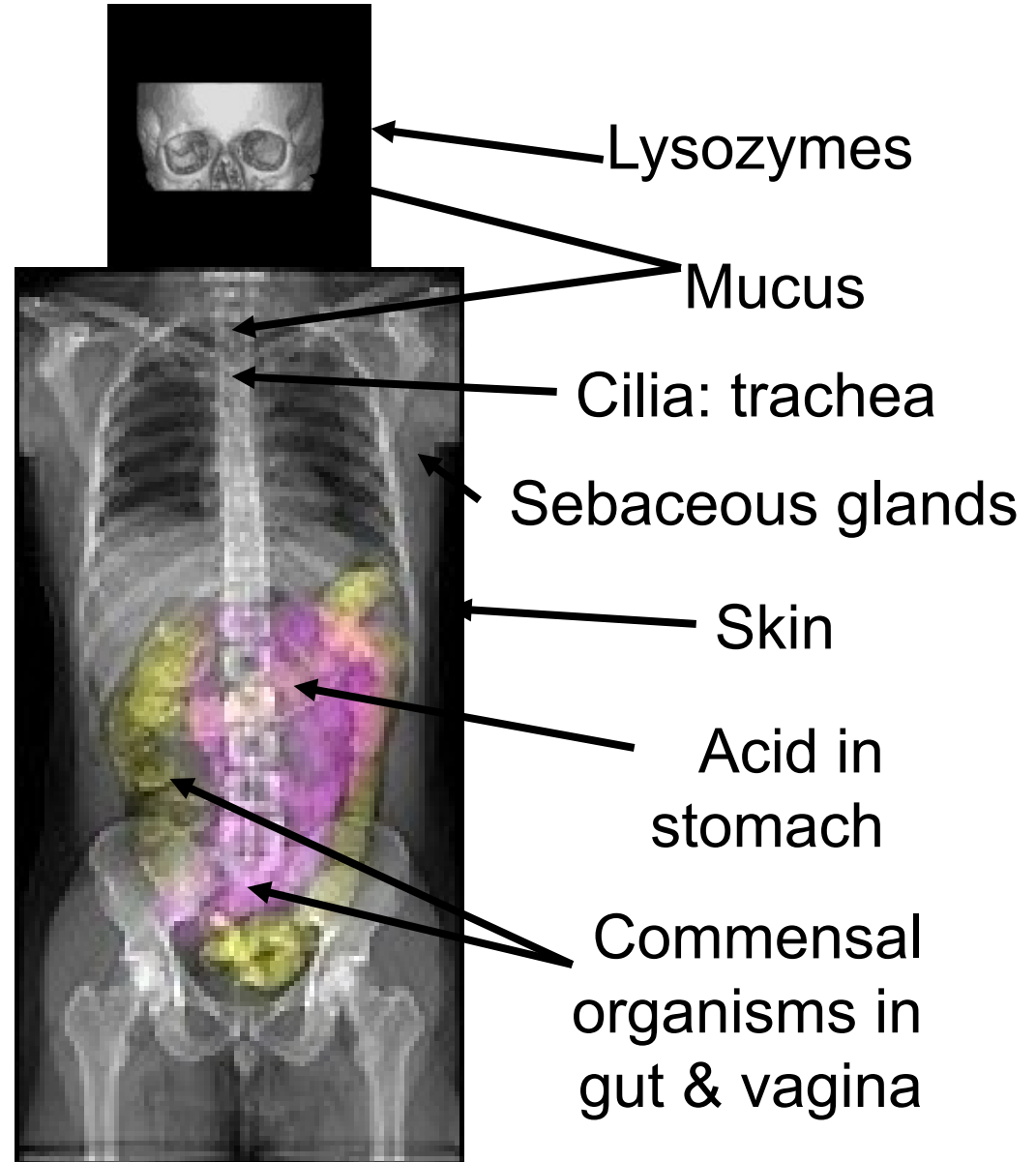
- enzymes, etc.
- secretions
- pH

b. Physical

- skin
- cilia

c. Cells

- Phagocytes, NK



The Innate immunity

- Inflammatory response: Overall non-specific reaction of the body to injury or invasion – starts immediately with infection or trauma.
 - Reactants may initiate, expand, or sustain the response
 - Can be acute (short duration) or become chronic (prolonged duration)
- Has 4 cardinal signs: **heat, pain, redness, edema** and as a result **loss of function** resulting from:

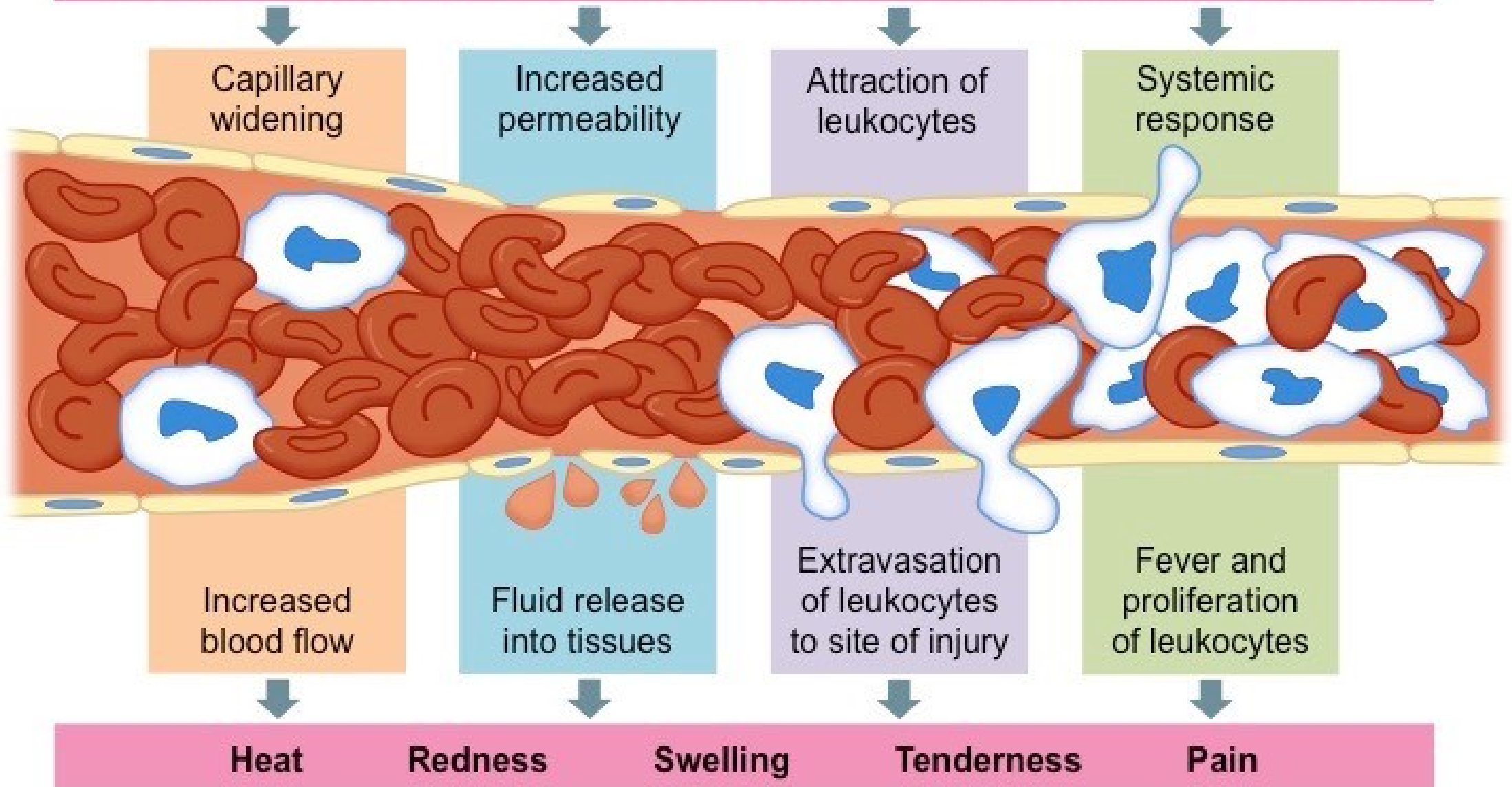
The Innate immunity

- Increased **blood and plasma flow** to the area
- Increased **capillary permeability** by retraction of endothelial cells
- mediated by vasoactive agents such as **histamine and prostaglandins**.
 - derived from injured cells and later from cells that infiltrate the area.
- Migration of leucocytes, particularly Neutrophils and macrophages, from the capillaries to the site of injury is due to a process called *chemotaxis*.

The Innate immunity

- Migration of white cells, especially early migration of neutrophils then macrophages to the area
- Increased release of mediators such as histamine from damaged mast cells – furthering capillary dilation
- Increased concentration of acute phase reactants that can amplify and/or control the response

Tissue injury caused by physical / chemical agent or pathogenic microorganism



The Innate immunity

1. External Innate Defense Systems

- Prevent entrance:
 - **Structural** barriers – effective with most microorganisms
 - **Skin** - epidermis = layers of tightly packed epithelial cells. Outer layer is dead cells and keratin, waterproofing protein
 - **Inner layer skin** - dermis = blood vessels, hair follicles, sweat glands, and sebaceous glands that produce an oily secretion called **sebum**
 - **Cilia** and **cough reflex** – helps expel microbe containing mucous
 - Sneeze

The Innate immunity

1. External Innate Defense Systems

- **Mucus** - conjunctivae, alimentary, respiratory, and urogenital tracts
 - saliva, tears, and mucous secretions wash away invaders and contain antibacterial or antiviral substances.
 - acidity (pH 5.6) of sweat, sebaceous glands, vagina (pH 5) and stomach (pH 1) – unfriendly to many microorganisms
 - enzymes present in the skin and stomach, tears
- **Normal flora** - out compete pathogens for attachment sites on the epithelial cell surface and for necessary nutrients.

The Innate immunity

2. Internal Innate Defense System

- To prevent the expansion of penetration
 - Recognize carbohydrates not normally present on cells such as mannose
 - May cause nonspecific activation of white cells
 - Phagocytosis – by neutrophils, eosinophils, basophils, or macrophages, mast cells, and dendritic cells
 - Clotting mechanism which entraps organisms in fibrin clots
 - Complement System can lyse cells or enhance phagocytosis

The Innate immunity

Physiologic Barriers

- Soluble factors contribute to innate immunity, they are collectively known as **acute phase reactants**.
- Normal serum components, non-specific responders to inflammation
- Increase because of infection, injury, trauma
- Produced mostly by the liver in response to inflammation and cytokine stimulation
 - Cytokines: IL-1, IL-6 and TNF alpha which are produced by macrophages and monocytes at the inflammatory site are activators

The Innate immunity

- **Complement** – a series of enzymes normally circulating in an inactive form
 - May be activated by the classical or alternate pathways
 - Can result in lysis or enhanced phagocytosis of cells
- **Lysozyme**, a hydrolytic enzyme in mucous secretions and in tears, can cleave the peptidoglycan layer of bacterial cell wall.
- **Interferon**, proteins produced by virus-infected cells. Has many functions including ability to bind to nearby cells and induce a generalized antiviral state.

The Innate immunity

C-Reactive Protein

- Normally trace levels in serum
- **Early acute inflammation indicator:**
 - increases within 4-6 hrs. of infection or trauma
 - 100 to 1000 fold increase serum concentration
 - concentration drops rapidly in serum when stimulus removed
- Enhances opsonization, agglutination, precipitation, and classical pathway complement activation – enhances the removal of irritant

The Innate immunity

Phagocytosis

- Phagocytic cells Chemotaxins such as
 - Complement components
 - Coagulation cascade proteins
 - Bacterial and viral products
- Attract phagocytic cells including:
 - Mast cell, lymphocyte, macrophage, neutrophil products
- Physical contact between the phagocytic cell and foreign object results in
 - Formation of phagosome
 - Formation of phagolysosome
 - Digestion
 - Release of debris

The Innate immunity

- **Phagocytosis**
 - Is a form of endocytosis.
 - Important body defense mechanism is process in which specialized cells engulf and destroy foreign particles such as microorganisms or damaged cells.
 - Macrophages and segmented Neutrophils are the most important phagocytic cells.

The Innate immunity

- **Phagocytosis ...**
 - **Adherence** – binding of an organism (PAMPs) to the surface of the phagocytic cell (PRR)
 - **Engulfment**:- is the ingestion and formation of phagosomes.
 - **Digestion** – after the foreign particle is ingested, cytoplasm lysosome fuse with phagosome The enzymes of lysosome then contribute to microbial killing and lysis.

...cont. Phagocytosis

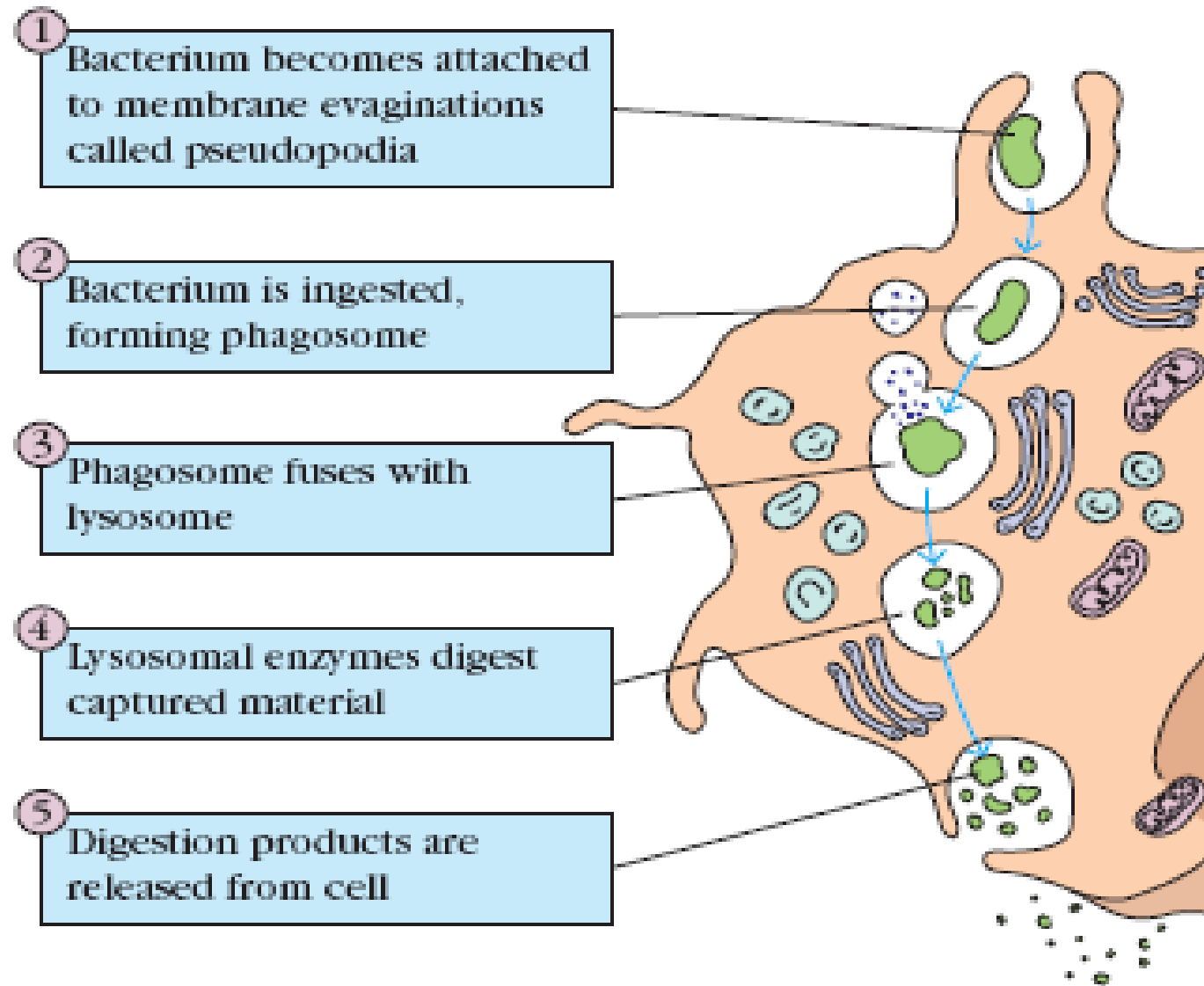
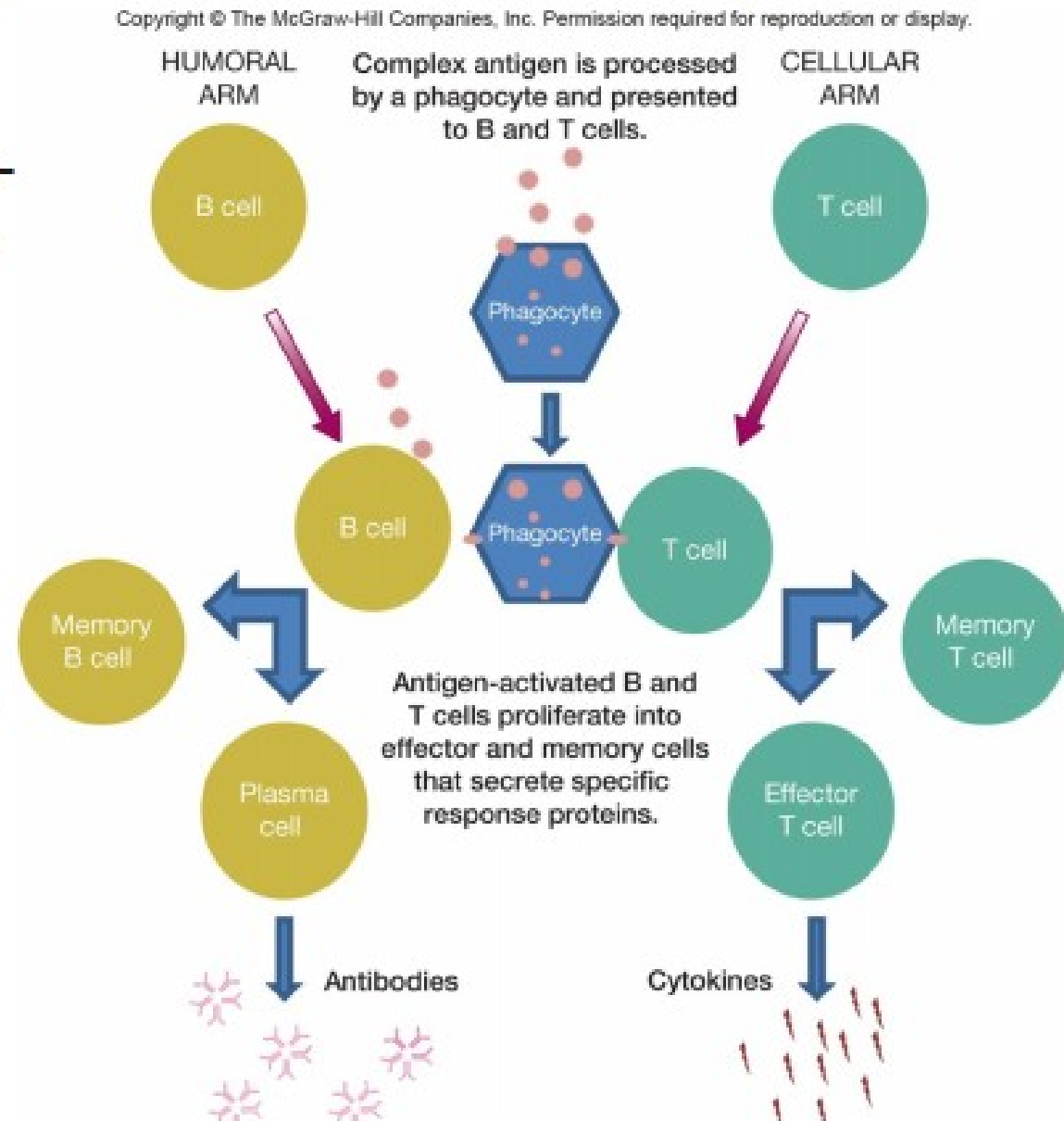


Table 1.1 Innate Defensive Components

Component	Effectors	Function
Anatomic and physiologic barriers	Skin and mucous membranes	– Physical barriers to limit entry, spread and replication of pathogens
	Temperature, acidic pH, lactic acid	
	Chemical mediators	
Inflammatory mediators	Complement	– Direct lysis of pathogen or infected cells
	Cytokines and interferons	– Activation of other immune components
	Lysozymes, defensins	– Bacterial destruction
	Acute phase proteins and lactoferrin	– Mediation of response
	Leukotrienes and prostaglandins	– Vasodilation and increased vascular permeability
Cellular components	Polymorphonuclear cells • Neutrophils, eosinophils • Basophils, mast cells	– Phagocytosis and intracellular destruction of microorganisms
	Phagocytic–endocytic cells • Monocytes and macrophages • Dendritic cells	– Presentation of foreign antigen to lymphocytes

Types of specific immunity

- **Humoral immunity**
 - also called antibody-mediated immunity
 - based on antibody activity
- **Cellular immunity**
 - also called cell-mediated immunity
 - based on action of specific kinds of T lymphocytes



Differences between Humoral and cell Mediated Immunity

	Humoral Immunity	Cell Mediated Immunity
Main cells involved	B lymphocytes	T lymphocytes
Where do cells develop?	Produced and mature in the bone marrow	Produced in the bone marrow, mature in the thymus gland
Antibodies?	Involves production of antibodies	Does not involve production of antibodies
How are pathogens identified?	Via antigens floating in the blood	Via antigens on the surface of infected cells.
How are pathogens killed?	By antibodies	By specialised 'killer T cells'
How do cells divide once they are stimulated?	cells divide into either plasma cells or memory cells	cells divide into different types of specialist T cells

Types of specific (adaptive, acquired) immunity

- Naturally acquired active immunity
 - the type of specific immunity a host develops after exposure to foreign substance
- Naturally acquired passive immunity
 - transfer of antibodies, e.g., mother to fetus across placenta, mother to infant in breast milk
- Artificially acquired active immunity (vaccination)
 - intentional exposure to a foreign material
- Artificially acquired passive immunity
 - preformed antibodies or lymphocytes produced by one host are introduced into another host

Acquired Immunity

Natural immunity

is acquired through the normal life experiences of a human and is not induced through medical means.

Active immunity

is the consequence of a person developing his or her own immune response to a microbe.



Infection

Passive immunity

is the consequence of one person receiving preformed immunity made by another person.



Maternal antibody

Artificial immunity

is that produced purposefully through medical procedures (also called immunization).

Active immunity

is the consequence of a person developing his or her own immune response to a microbe.



Vaccination

Passive immunity

is the consequence of one person receiving preformed immunity made by another person.



Immune globulin therapy

Characteristics of adaptive immunity

- Characteristics of the acquired immune response:
 - 1- Self/Non-self recognition: T and B cells have the ability to recognize self from non-self cells
 - 2- Specificity: The ability to discriminate among different molecular entities and to respond only to those uniquely required because of great diversity of B and T cells
 - 2- Diversity: There are potentially billions of different antigen receptors on B cells and T cells that recognize different antigens
 - 4- Memory: the ability to recall previously contacted antigens.

The response here takes place in a learned manner (more rapid and larger response) anamnestic response.

Innate immunity vs Adaptive Immunity

Innate Immunity (first line of defense)	Adaptive Immunity (second line of defense)
• No time lag • Not antigen specific - No memory	• A lag period • Antigen specific • Development of memory

Cells of the adaptive immune system

Three types of cells are involved in acquired immunity:

- The three types of cells need to interact and co-operate with each other to achieve a full successive immune response.
- Two type of cells rise from the same lymphoid stem cell (precursor). But they differentiate along different developmental lines. One of these cells develops in the **thymus** to give **T cells** and the other develops in the **bone marrow** to give **B cells**.
- The third type of cells referred to as **antigen-presenting cell APC**. APC include macrophages or dendritic cells.
- APC does NOT recognize antigens specifically, their importance comes from antigen processing and presenting.
- The presentation of antigen takes place by surface receptors; major histocompatibility complex, MHC-I and MHC-II.

Innate immunity



Basophil



Eosinophil



Neutrophil



Mast cell



Natural killer cell



Macrophage



Dendritic cell

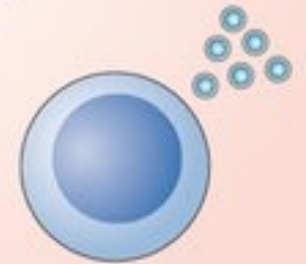
Adaptive immunity

Antibodies



B cell

Cytokines
Cytotoxic molecules



T cell

Antigen
presentation

Hours

Time of response

Days

Acquired immunity

Clonal selection Theory:

- Discuss the specificity of the immune response “ Can recognize self from non-self antigens”
- Specificity depends on the components of the immune system which are capable of recognizing and reacting to Ag.
- These components include T and B lymphocytes.
- The theory **eliminates** self-reacting lymphocytes.

Basis of clonal selection theory:

- 1- T and B lymphocytes exhibit specificity to Ag before coming intact with the Ag.
 - 2- T and B lymphocytes express specific receptors on their surfaces for that particular Ag.
- After T and B lymphocytes recognize and bind Ag

Acquired immunity

- They secrete specific molecules. B-cells secrete Antibodies whereas T-cells secrete Cytokines.
 - B-cell recognise Ag by antibody molecule found on its surface.
 - T-cell recognise Ag presented to it by MHC-I or II by a specific receptor on its surface T cell receptors (TCRs).
- 3- Each cell holds one single receptor on its surface.
- 4- T and B lymphocytes bind Ag on a particular site which is specific to the Ag (epitope).
- After binding and activation T and B- cells secrete a vast range of cytokines lead to proliferation and differentiation of the same clone of cells.
- 5- introduction of self Ags in early stages of development (education) to avoid self Ag reaction

Acquired immunity

Humoral and Cellular immunity:

Humoral immunity mediated with B lymphocytes and antibodies secretion (immunoglobulins) Igs.

- There are many classes of Igs (IgG, IgM, IgA, IgD and IgE).
- Ag/Ab binding should activate other serum components that lead to the destruction of Ag.

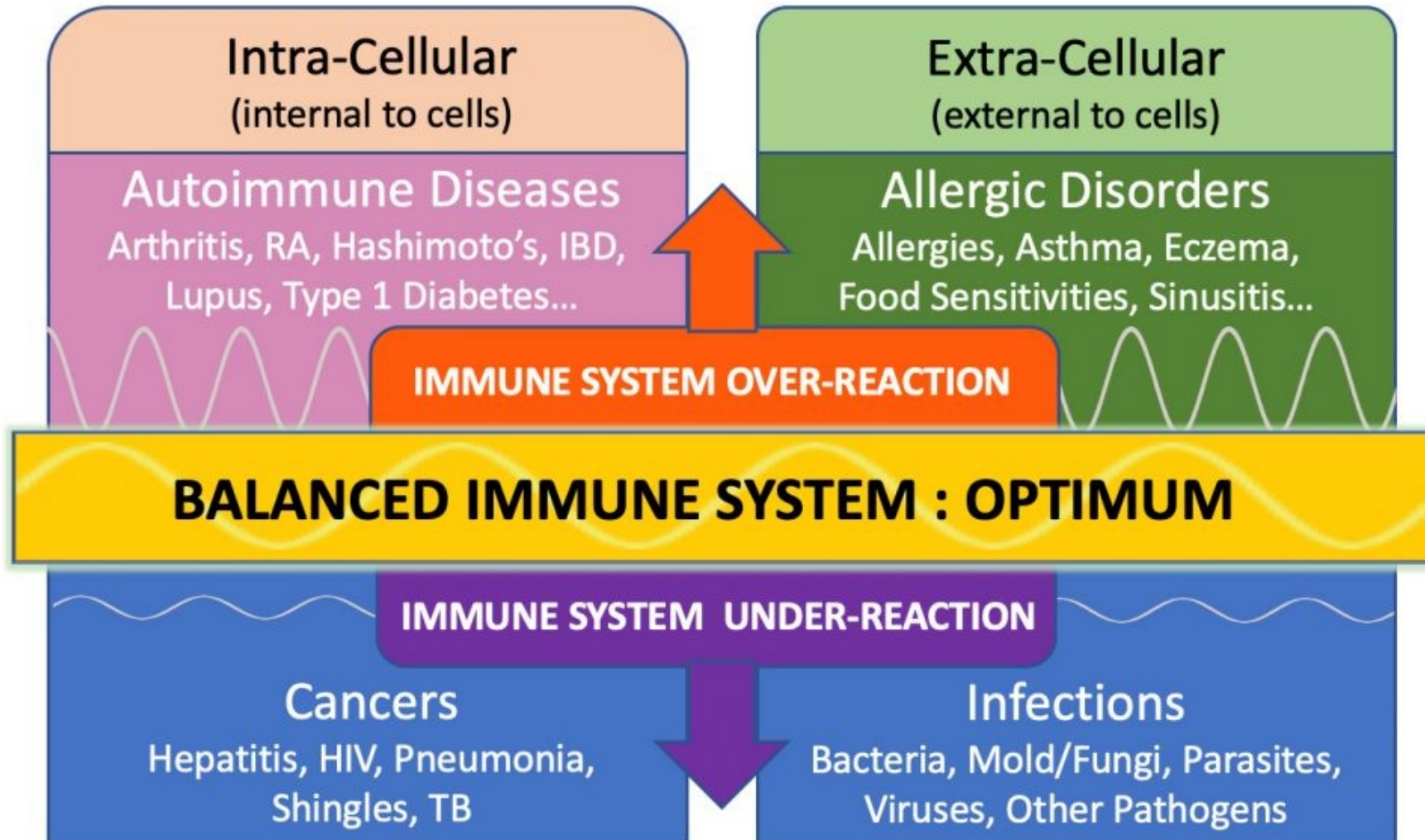
Cellular immunity mediated with T lymphocytes and molecules secreted in order of B lymphocytes activation and secrete antibodies (Abs).

Acquired immunity

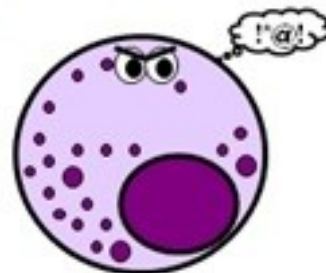
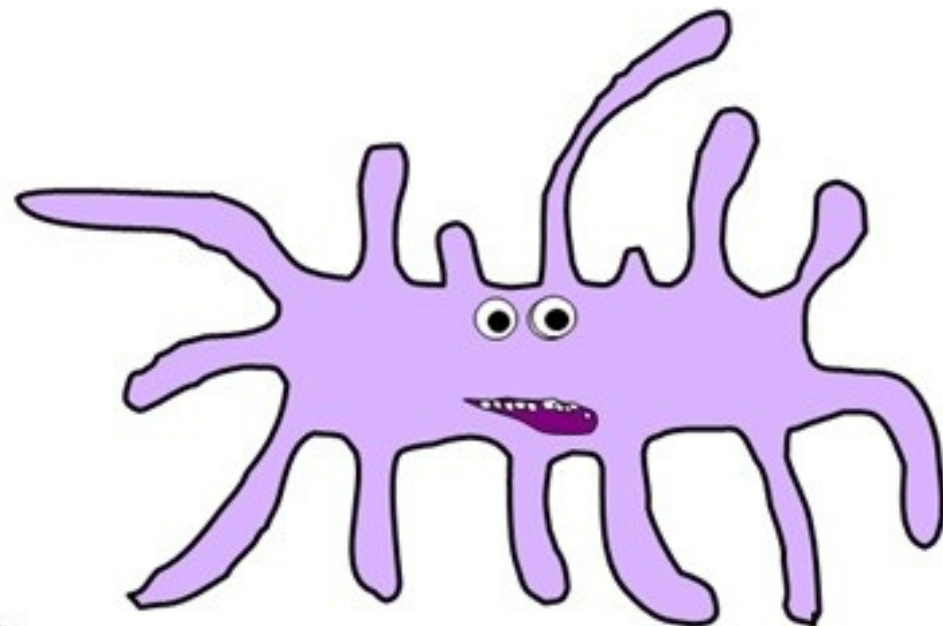
- T lymphocytes NEVER binds Ag directly.
- T lymphocytes recognise Ag only if presented by MHC-I and II. As there are two classes of MHC molecules there are more than one T lymphocytes subpopulation.
- T-lymphocytes have T-helper cells (TH) and T- cytotoxic (TC).
- (TH) also called CD4 cell and (TC) CD8 cell.

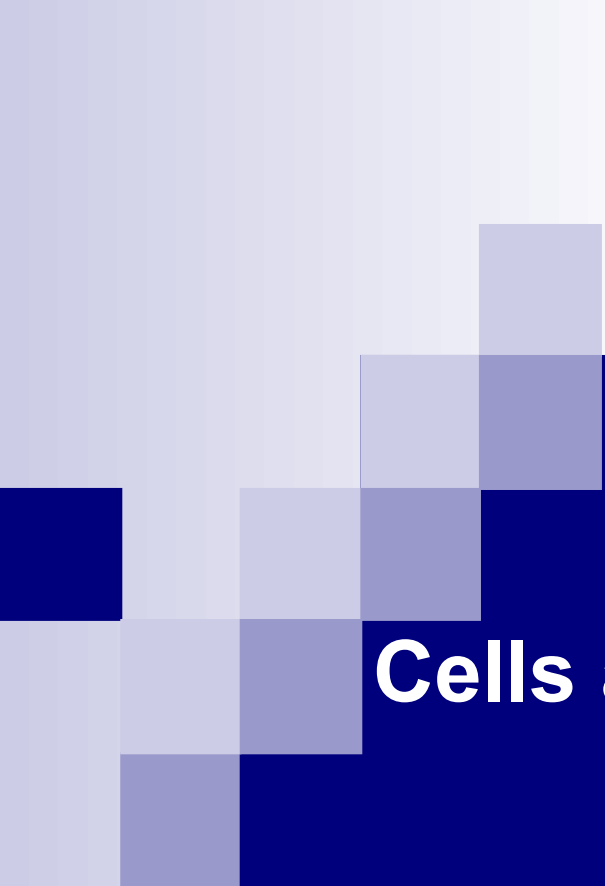
IMMUNE SYSTEM

Balance is better than Boost



Good luck!





CHAPTER 2

Cells and organs of immune system

Learning Objectives

Upon completion of this lesson the student will be able to:

- Describe cells and organs of immune system
- Describe lymphoid tissue by primary or secondary, locations of specialized tissues, cells produced and key role in immunity.
- Describe the morphology, source and role of macrophages, natural killer cells, cytotoxic, helper, suppressor or B lymphocytes and plasma cells.
- Discuss the role of surface markers in cells involved with immunity, referring to specific markers used to differentiate T and B lymphocytes.

Outline

2.1. Organs of the immune system

2.2. Cells of the immune system

2.1. Organs of the immune system

Introduction

- The immune system consists of many different organs and tissues that are found throughout the body.
- These organs can be classified functionally into two main groups.
 - The primary lymphoid organs provide appropriate microenvironments for the development and maturation of lymphocytes. This includes Thymus, Bone marrow, Fetal liver

Cont...

- The secondary lymphoid organs trap antigen from defined tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with that antigen.
- Blood vessels and lymphatic systems connect these organs, uniting them into a functional whole.
- These are
 - Lymph nodes, Spleen,
 - Mucosa Associated Lymph tissue (**MALT**)
 - Tonsils, Peyers patches, lamina propria (largest amount of lymphs), appendix collectively known Gut associated lymphoid tissue (**GALT**)
 - Bronchial associated lymphoid tissue (**BALT**)

Cont...

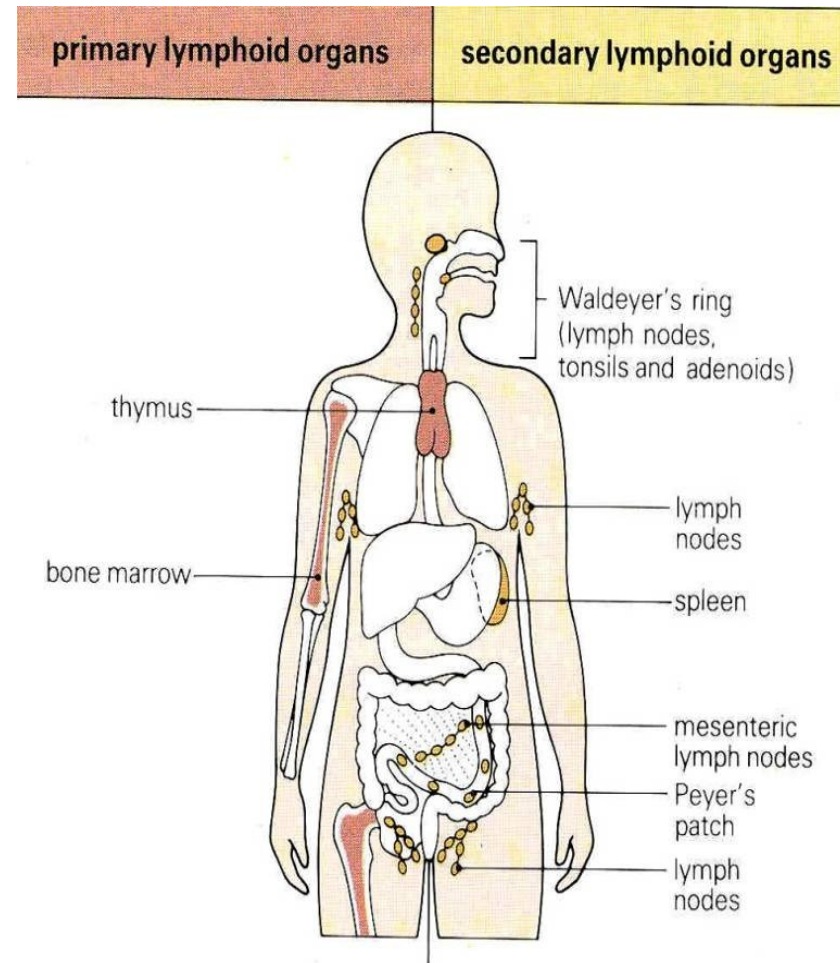
- Once mature lymphocytes have been generated in the primary lymphoid organs, they circulate in the blood and **lymphatic system**, a network of vessels that collect fluid that has escaped into the tissues from capillaries of the circulatory system and ultimately return it to the blood.

Cont...

Primary Lymphoid System

Classification-Residence

1. **Primary** lymphoid tissue
primary diff/maturation
 1. Thymus
 2. Bone marrow
 3. Fetal liver
2. **Secondary** Lymphoid tissue
- Ag exposure and final differentiation
 1. Lymph nodes, Spleen
 2. Mucosa Associated Lymph tissue (MALT)



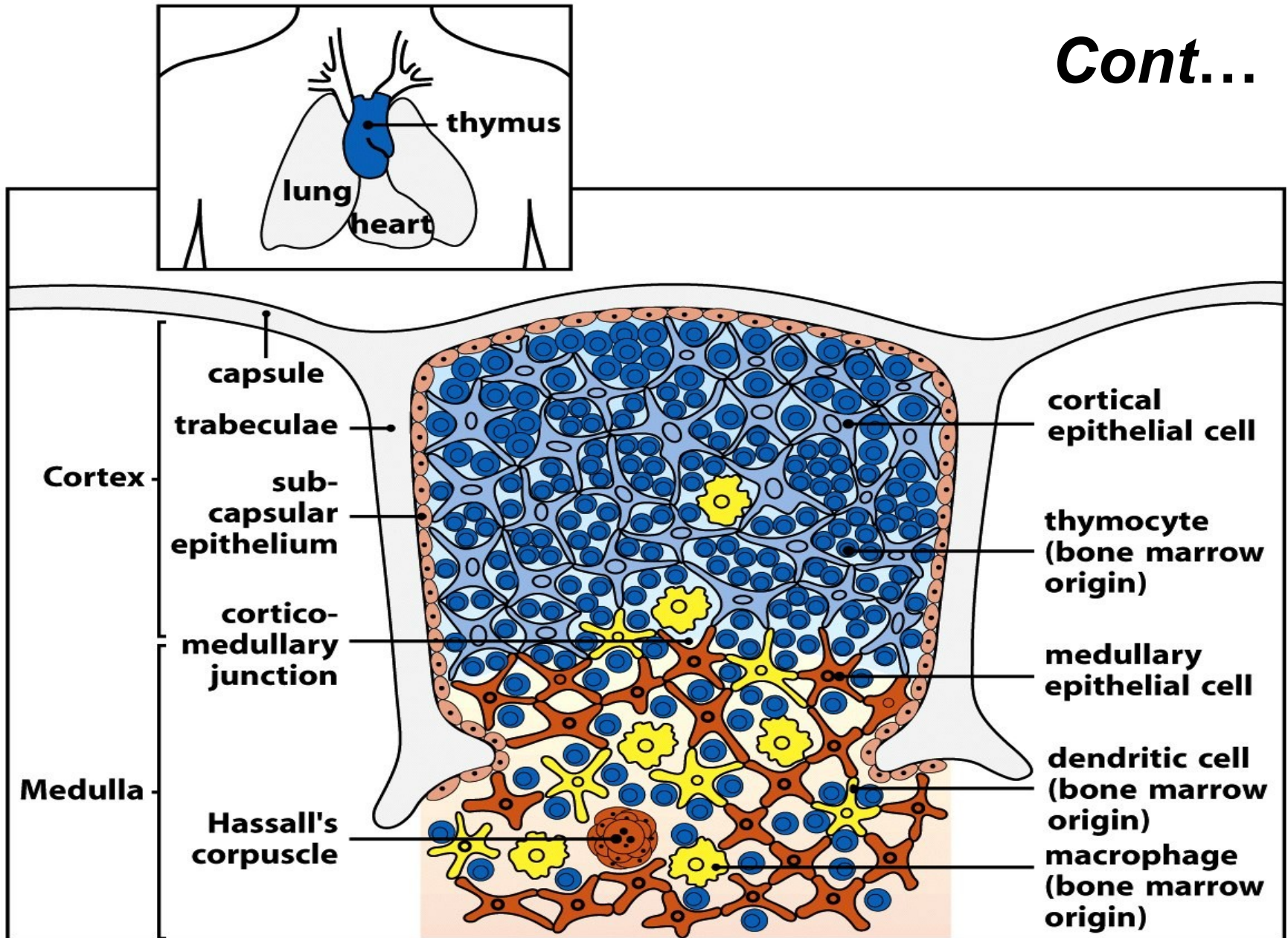
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Cont...

Thymus

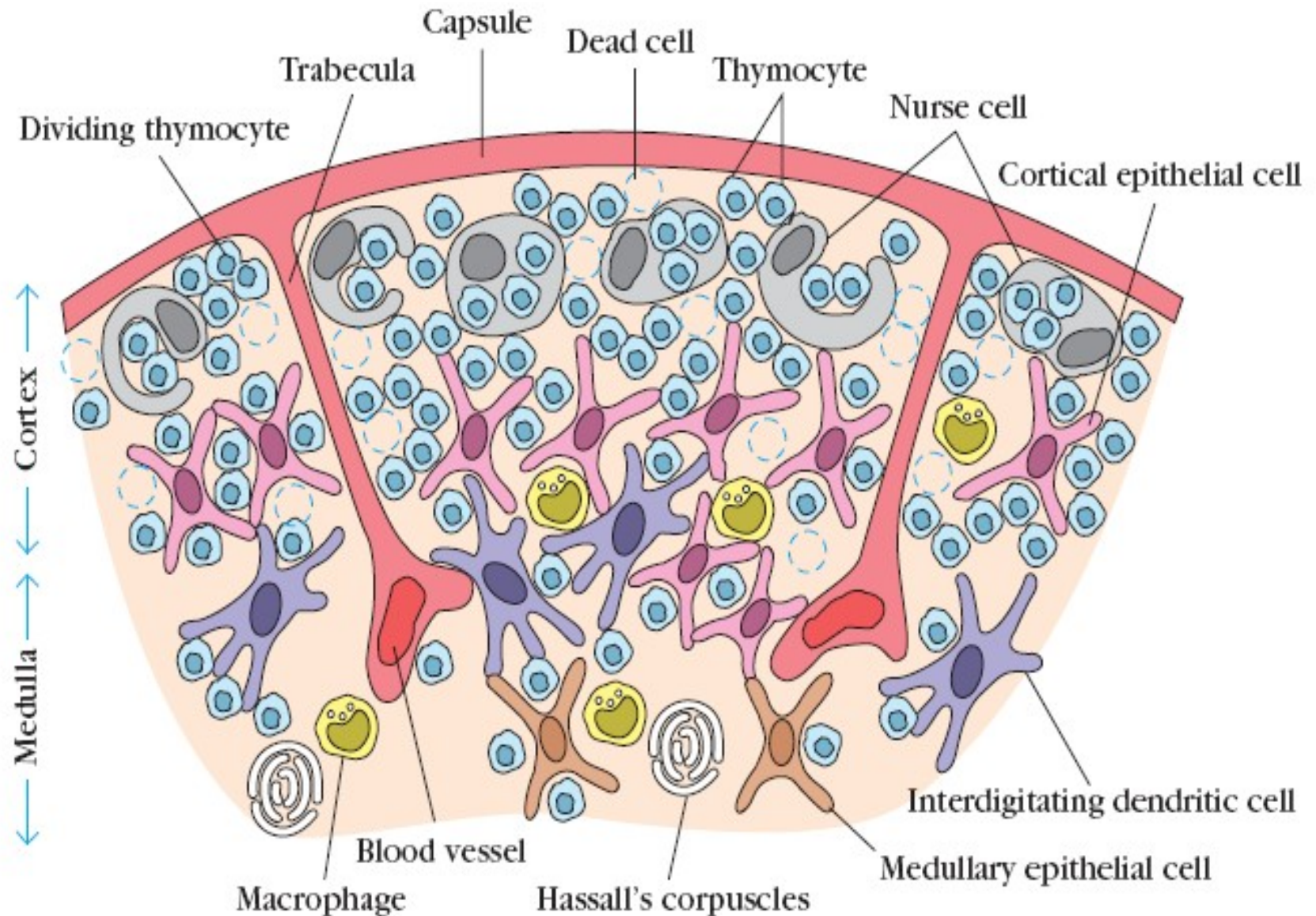
- the site of T-cell development and maturation.
- flat, bilobed organ situated above the heart.
- each lobe is surrounded by a capsule and is divided into lobules.
- each lobule is organized into two compartments:
- the **outer** compartment, or *cortex*, is densely packed with immature T cells, called thymocytes.
- the **inner** compartment, or *medulla*, is sparsely populated with thymocytes.

Cont...



Source: Kuby immunology 2007, 5th ed

Cont...



Source: Kuby immunology 2007, 5th ed

Cont...

- Both the cortex and medulla are crisscrossed by a three-dimensional stromal-cell network composed of
 - epithelial cells,
 - dendritic cells, and
 - macrophages, which make up the framework of the organ and contribute to the growth and maturation of thymocytes (T cells)

Cont...

Function of Thymus

- to generate and select a repertoire of T cells that will protect the body from infection.
- As thymocytes develop, an enormous diversity of T-cell receptors is generated by a random process that produces some T cells with receptors capable of recognizing antigen-MHC complexes.

Cont...

- Most of the T-cell receptors produced by this random process are incapable of recognizing antigen-MHC complexes and a small portion react with combinations of self antigen-MHC complexes.
- More than 95% of all thymocytes die by apoptosis in the thymus without ever reaching maturity.

Cont...

Bone Marrow

- The site of B-cell origin and development.
- Immature B cells proliferate and differentiate within the bone marrow, and stromal cells within the bone marrow interact directly with the B cells and secrete various cytokines that are required for development.
- Selection process within the bone marrow eliminates B cells with self-reactive antibody receptors.

Cont...

Lymph Nodes

- These are sites where immune responses are mounted to antigens in lymph.
- Are encapsulated bean shaped structures containing a reticular network packed with
 - lymphocytes,
 - macrophages, and
 - dendritic cells

Cont...

Lymph Nodes

- The first organized lymphoid structure to encounter antigens that enter the tissue spaces.
- Morphology
 - lymph node can be divided into three
- The cortex
 - The outermost layer,,
 - Contains lymphocytes (mostly B cells), macrophages, and follicular dendritic cells arranged in primary follicles.
 - After antigenic challenge, the primary follicles enlarge into secondary follicles, each containing a germinal center.

Cont...

■ The **paracortex**

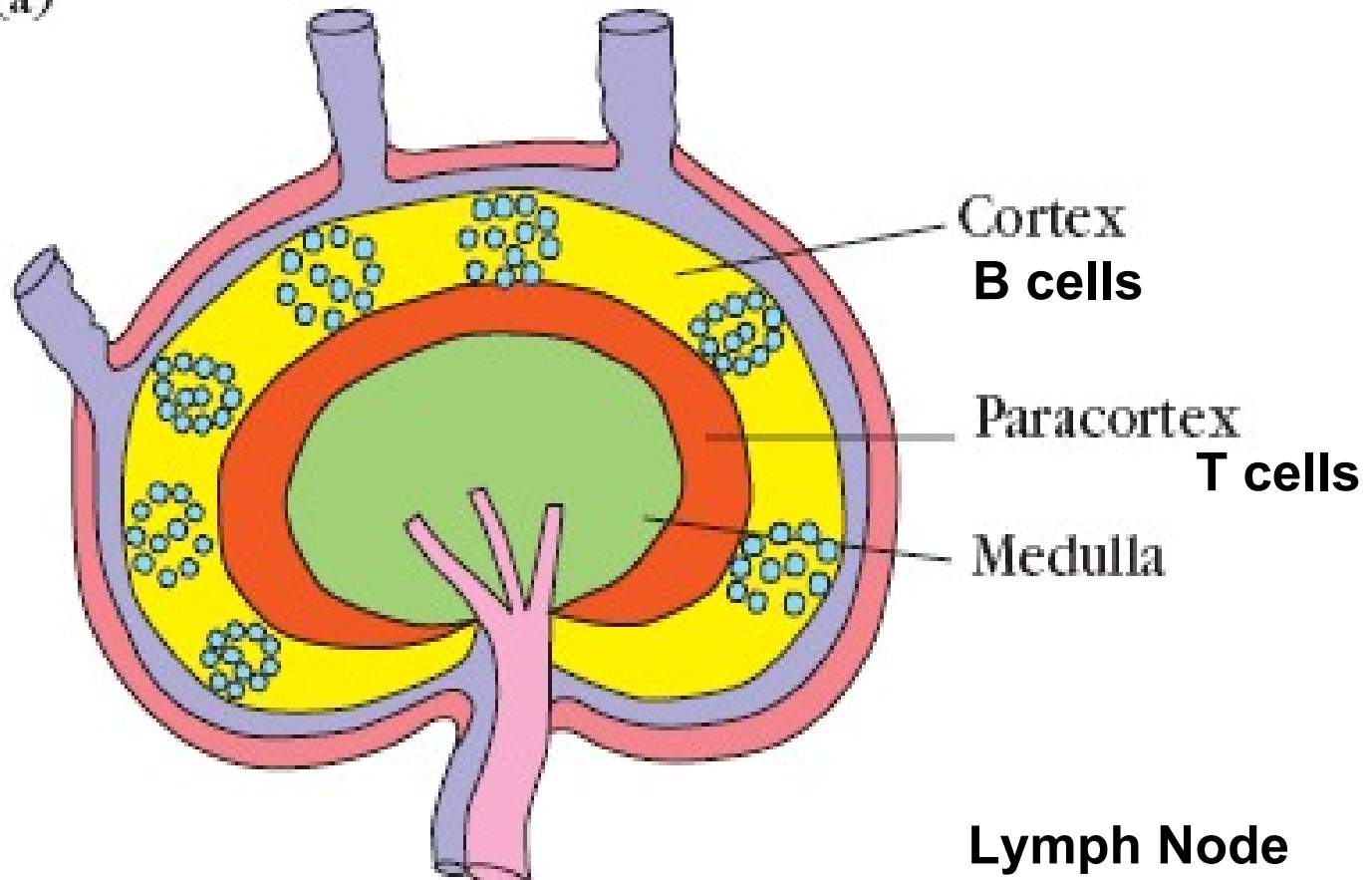
- Beneath the cortex.
- Populated largely by T lymphocytes and
- Contains interdigitating dendritic cells
- These interdigitating dendritic cells express high levels of class II MHC molecules, which function as APC.

■ The **medulla**

- The innermost layer .
- More sparsely populated with lymphoid-lineage cells; of those present, many are plasma cells actively secreting antibody molecules.

Cont...

(a)



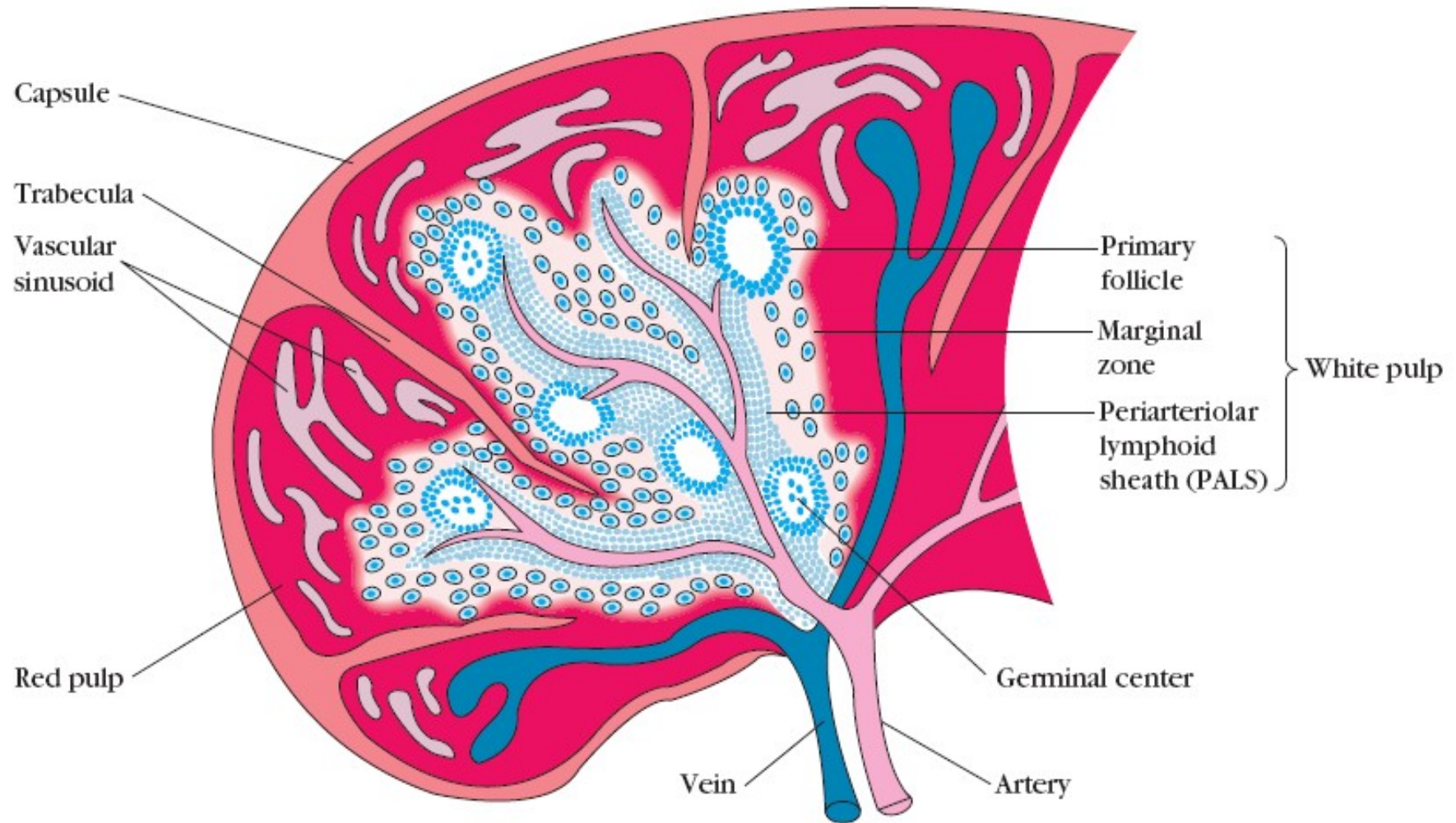
Source: Kuby immunology 2007, 5th ed

Cont...

Spleen

- Plays a major role in mounting immune responses to antigens in the **blood stream**.
- Large, ovoid situated high in the left abdominal cavity.
- Specializes in **filtering blood** and trapping **blood-borne antigens**;
- Can respond to systemic infections.
- Two compartments
 - The red pulp populated by Macrophages, RBCs and few lymphocytes
 - A site where old and defective RBCs are destroyed and removed
 - White pulp, is primarily populated by T cells and B cells

Cont...



Structure of SPLEEN

Source: Kuby immunology 2007, 5th ed

Cont...

Cutaneous-Associated Lymphoid Tissue

- The skin is a barrier to the external environment.
- Important in nonspecific defenses.
- Epithelial cells in the outer layer of the skin (keratinocytes) secrete a number of cytokines that may function to induce a local inflammatory reaction.

Cont...

- Keratinocytes can be induced to express class II MHC molecules and may function as APC.
- The Langerhans cells “immature DCs” migrate from the epidermis to regional lymph nodes, where they differentiate into interdigitating dendritic cells.
- These cells express high levels of class II MHC molecules and function as potent activators of naive TH cell

Summary

- The cells that participate in the immune response are white blood cells
- The lymphocyte is the only cell to possess the immunologic attributes of specificity, diversity, memory, and self/non self recognition
- The primary lymphoid organs provide sites where lymphocytes mature and become antigenically committed
- Secondary lymphoid organs capture antigens and provide sites where lymphocytes become activated by interaction with antigens.

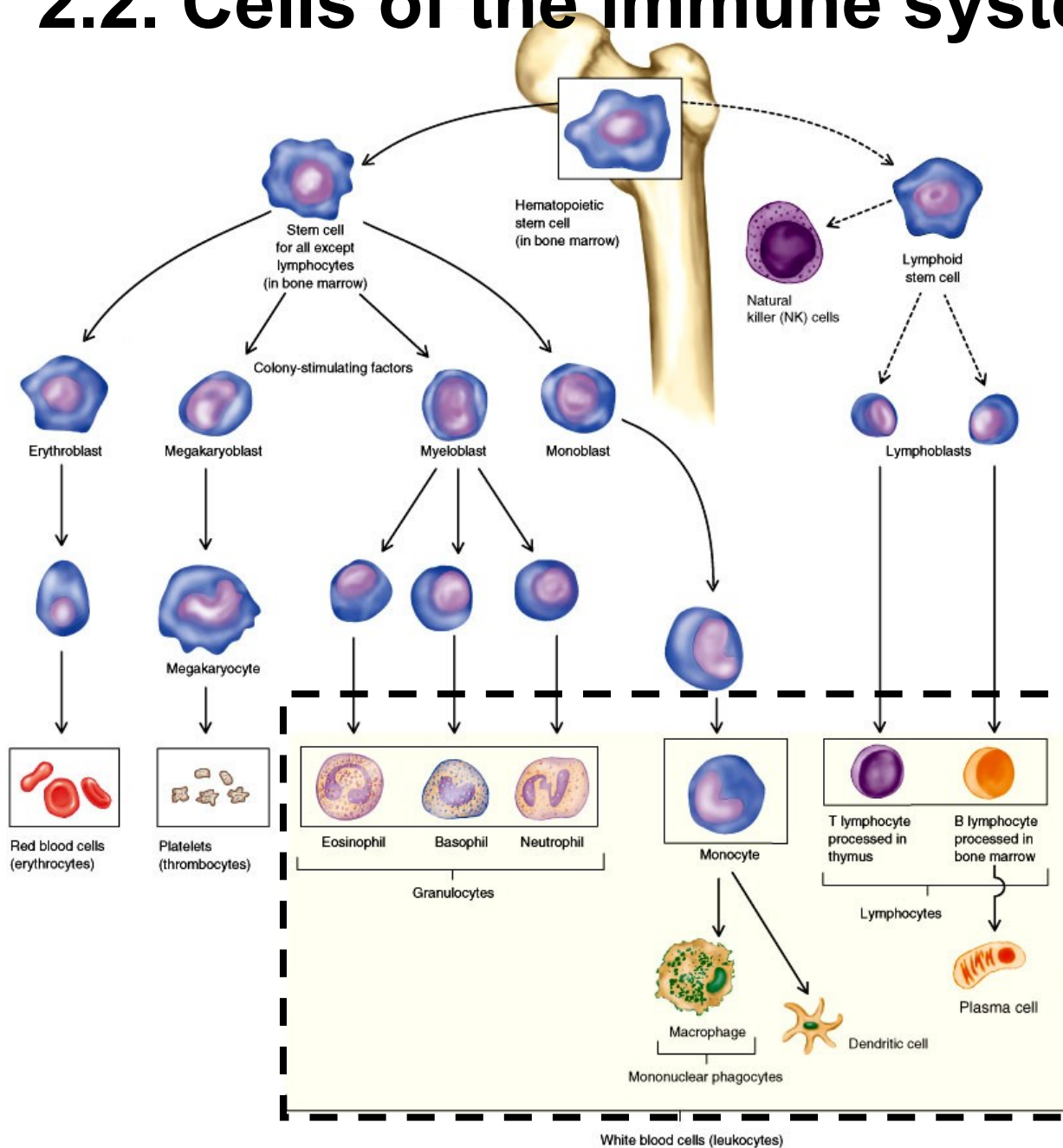
Development of Immune cells

Hematopoiesis

- All blood cells arise from a type of cell called the **hematopoietic stem cell (HSC)**.
- **Stem cells** are cells that can differentiate into other cell types; they are self-renewing-they maintain their population level by cell division.
- In humans, **hematopoiesis**, the formation and development of red and WBCs, begins in the embryonic yolk sac during the first weeks of development.

2.2. Cells of the immune system

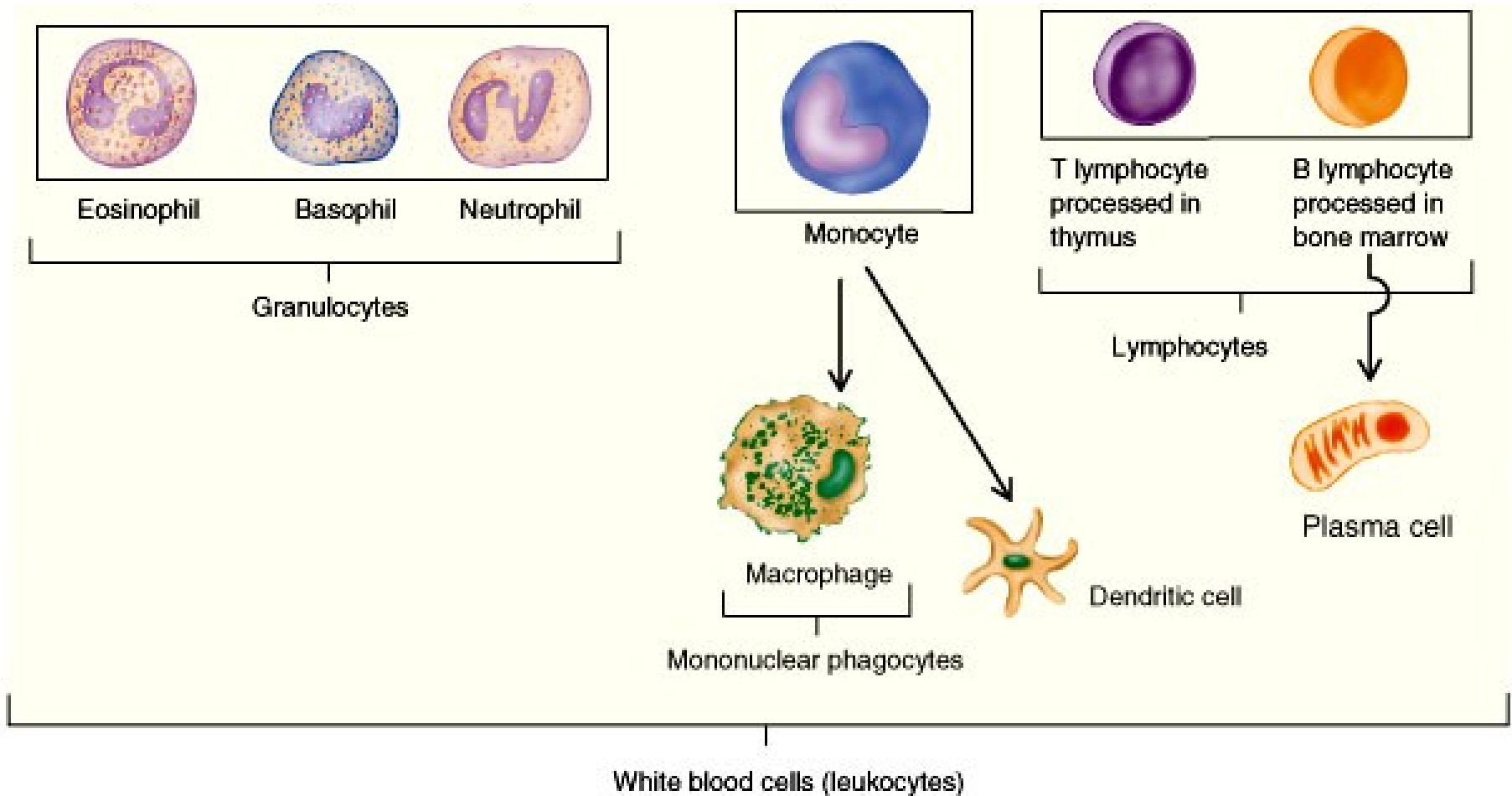
Development of immune Cells



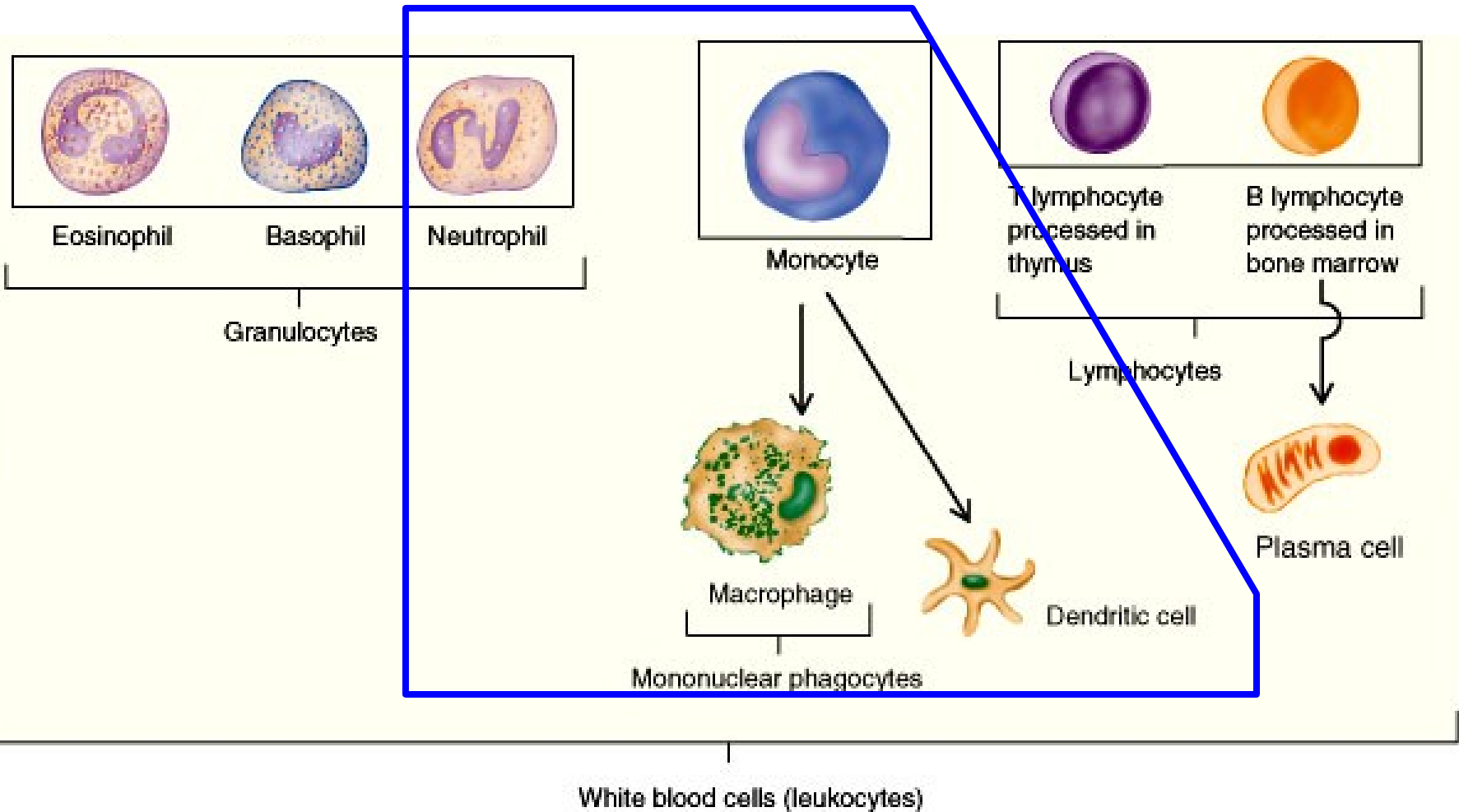
Normal Blood Cell Counts

	Mean Number (per mm ³)	Normal Range
White blood cells (leukocytes)	7400	4500– 11,000/mm ³
Neutrophils	4400	40%–60%
Eosinophils	200	1%–4%
Basophils	40	<1%
Lymphocytes	2500	20%–40%
Monocytes	300	2%–8%

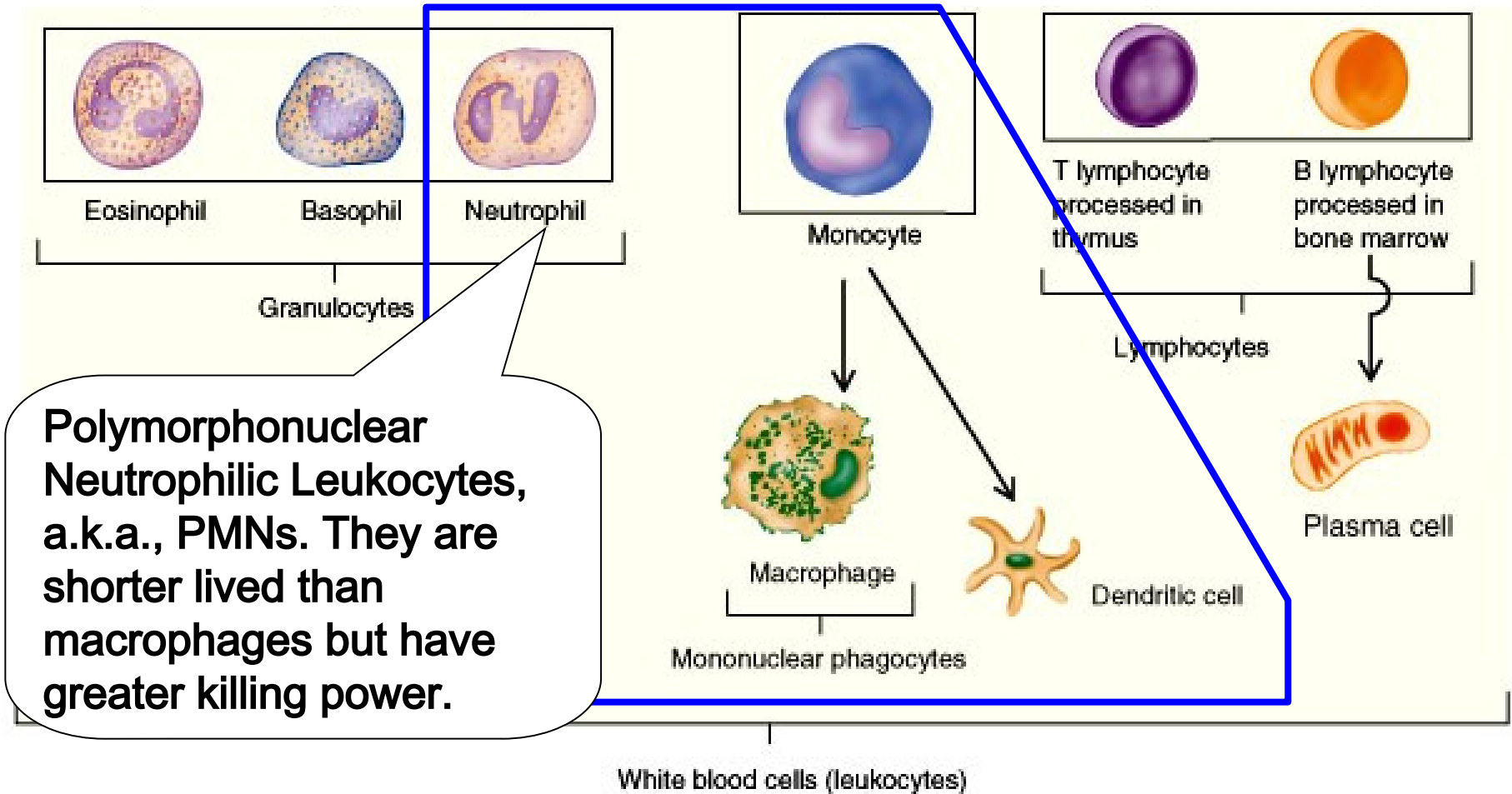
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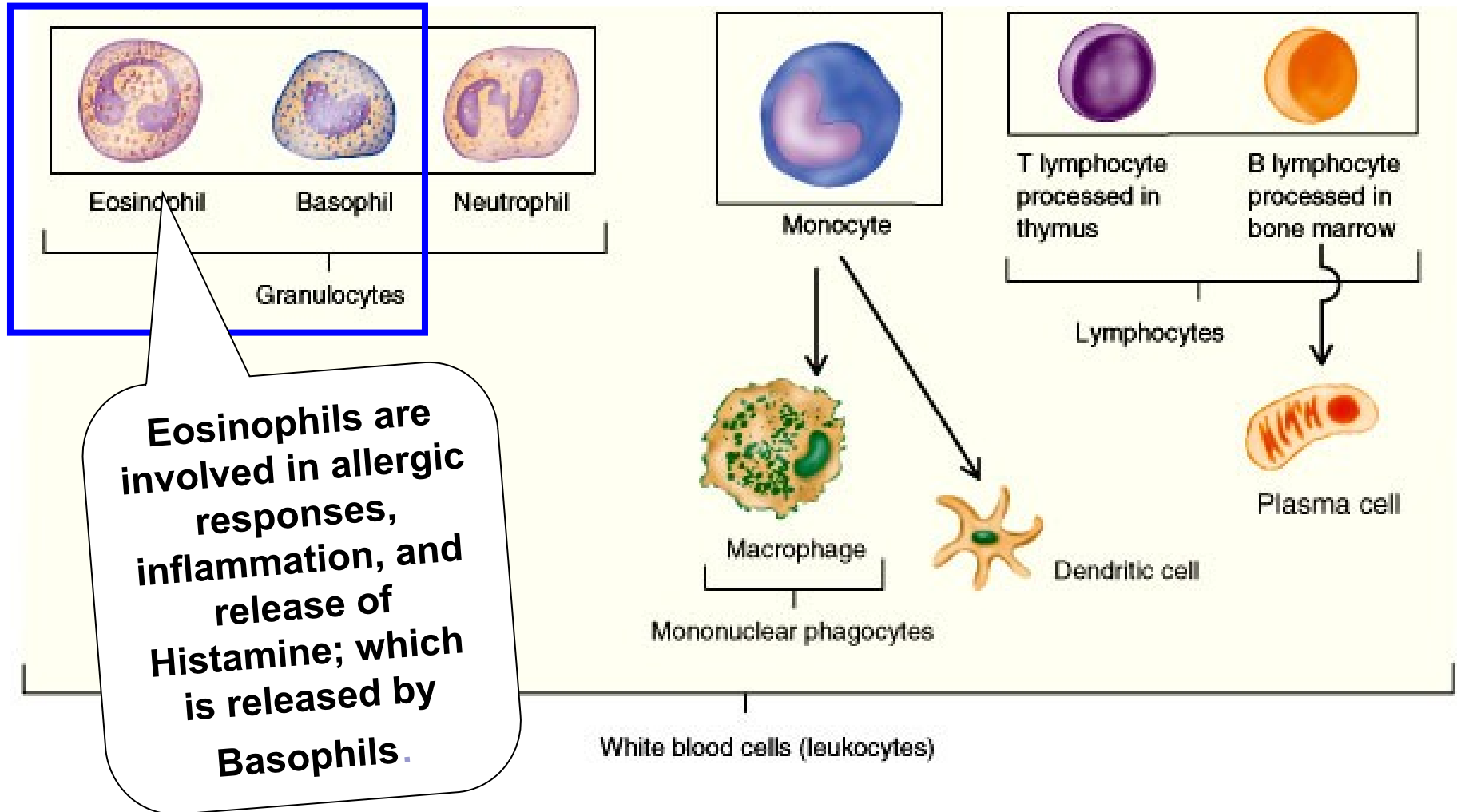
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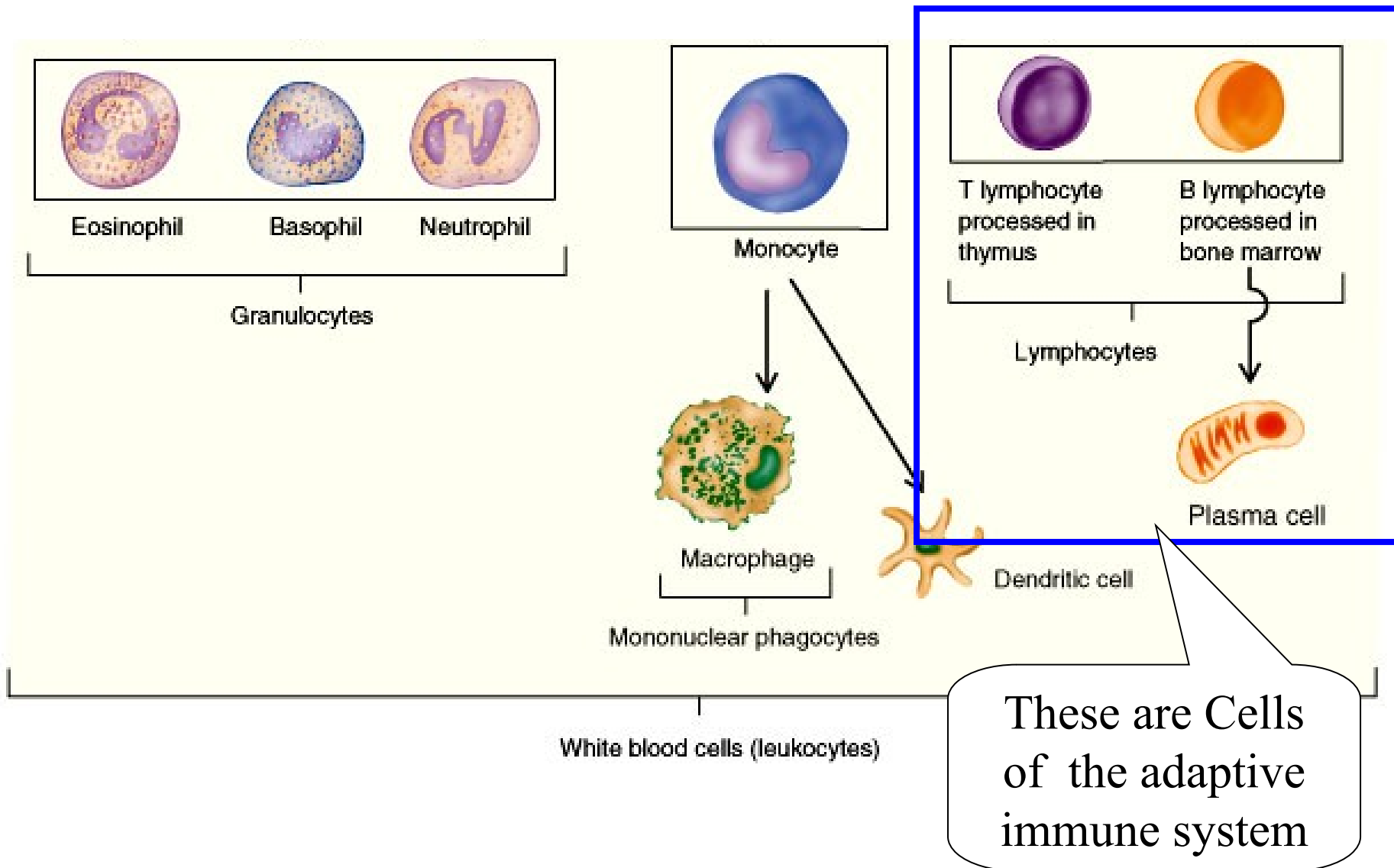
2.2. Cells of the immune system



2.2. Cells of the immune system



2.2. Cells of the immune system



2.2. Cells of the immune system

- Lymphocytes: (B cells and T cells): Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/non-self recognition.
- The other types of white blood cells play important roles, engulfing and destroying microorganisms, presenting antigens, and secreting cytokines such as Natural Killer cells (NKs), Neutrophils, Macrophage, Eosinophils, Basophils, Mast cells and Dendritic cells.

2.2. Cells of the immune system

Natural Killer Cells

- Natural killer cells (NKs), described in 1970s, were shown to be a small population of large, granular lymphocytes that display cytotoxic activity against a wide range of tumor cells.
- NK cells play an important role in host defense both against tumor cells and against cells infected with some though not all, viruses.

2.2. Cells of the immune system

NK Cells...

- Do not express the membrane molecules and receptors that distinguish T- and B-cell lineages.
- Although NK cells do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes, they can recognize potential target cells in two different ways:

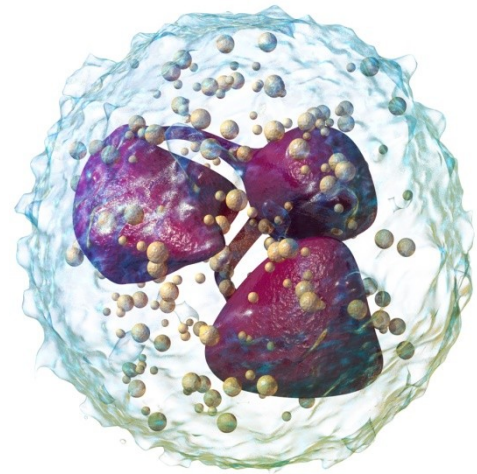
2.2. Cells of the immune system

NK cells...

- NK cells may employ receptors that distinguish abnormalities, notably a reduction in class I MHC molecules and the unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses.
- NK cells recognize potential target cells depends upon the fact that some tumor cells and cells infected by certain viruses display antigens against which the immune system has made an antibody response, so that antitumor or antiviral antibodies are bound to their surfaces

2.2. Cells of the immune system

- **Polymorphonuclear phagocytic cells (PMNs)**
 - PMNs are capable of ingesting and digesting exogenous antigens, such as whole microorganisms and insoluble particles.



2.2. Cells of the immune system

DCs...

- Derived from myeloid progenitor

Immature DCs:

- Migrate from blood to reside in tissues.
- Upon encountering a pathogen, they readily mature, express co-stimulatory molecules, and migrate to lymph nodes
- Example: Langerhans cells (epidermis/skin and mucous membranes)

2.2. Cells of the immune system

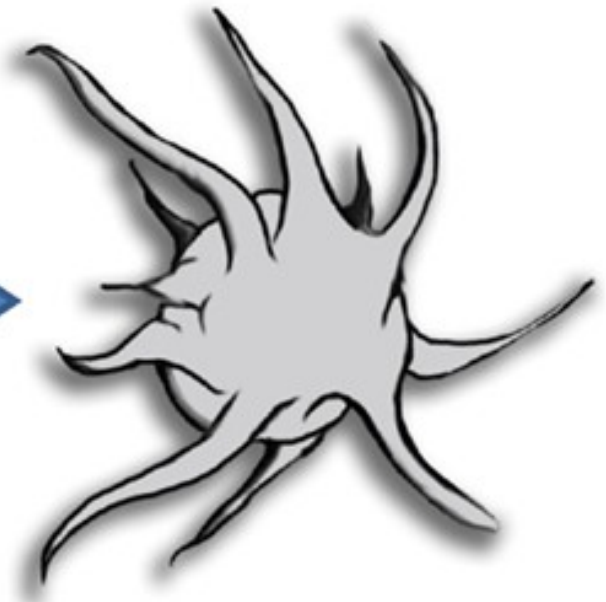
DCs...

Mature DCs:

- Specialized to take up Ag, process it, and display it for recognition by T Lymphocytes i.e., act as APCs to T cells initiating adaptive IR (express co-stimulatory molecules when encountering pathogen)
- Example:
 - Interstitial DCs (organs: heart, lungs, liver, kidney, GIT)

immature DC

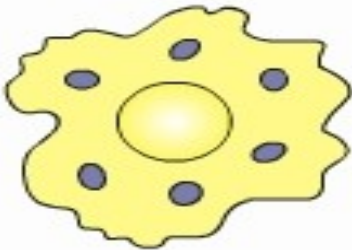
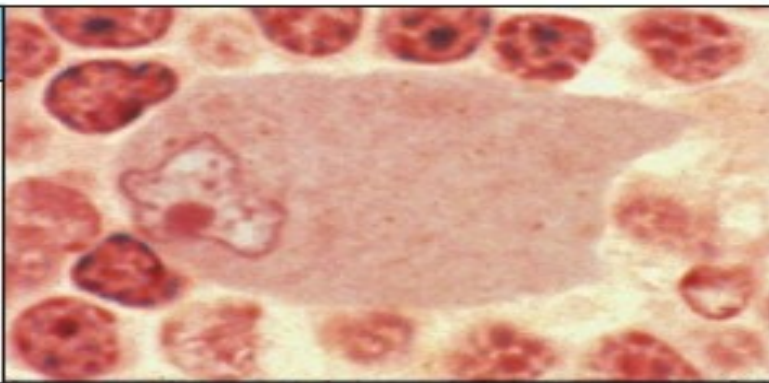
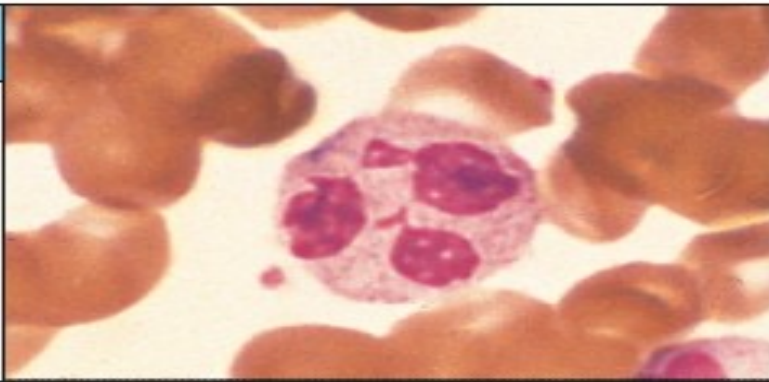
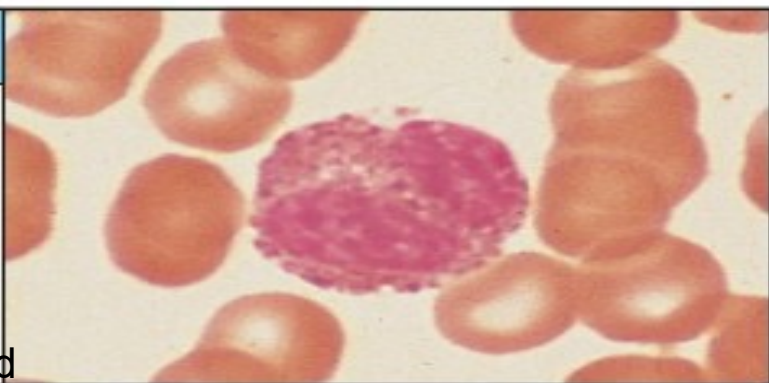
mature DC



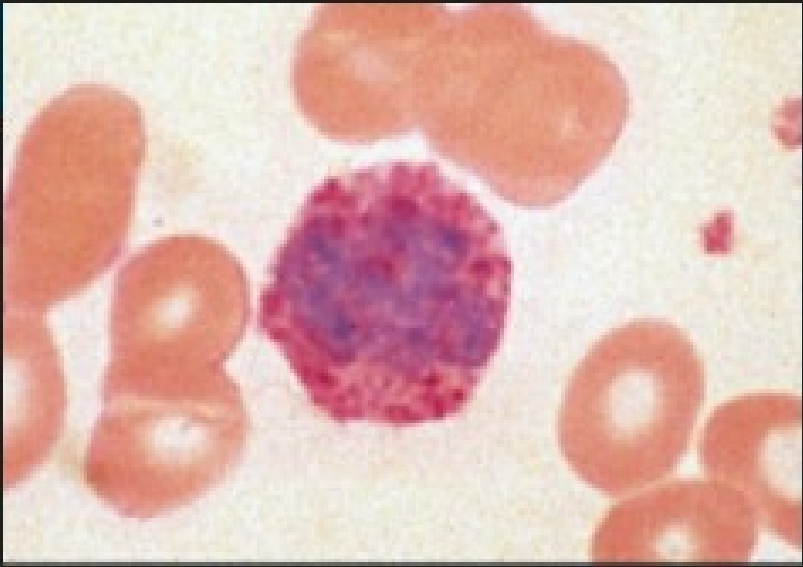
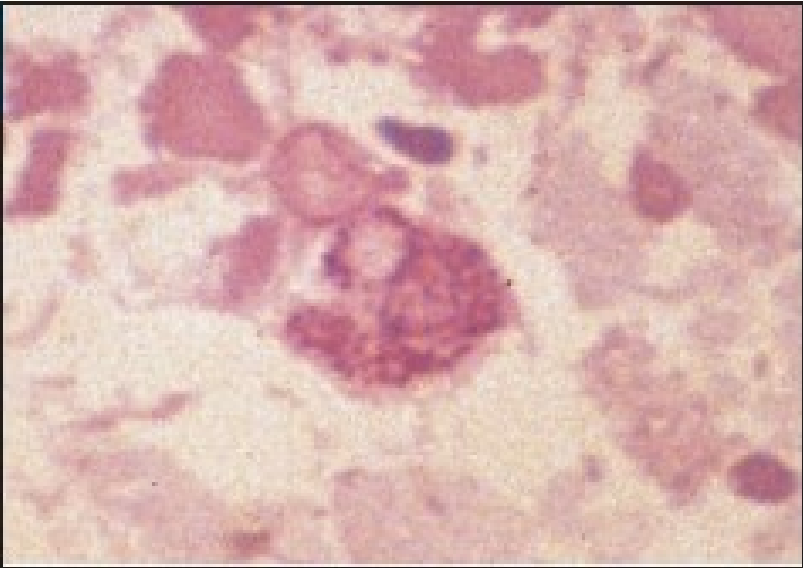
antigen uptake
antigen processing

antigen presentation
costimulation
T cell activation

2.2. Cells of the immune system

Cell	Activated function
Macrophage 	 Phagocytosis
Neutrophil 	 Phagocytosis and activation of bactericidal mechanisms
Eosinophil 	 Killing of antibody-coated parasites

2.2. Cells of the immune system

Basophil	 A microscopic image of a basophil, showing a large, round cell with a dark purple, granular nucleus and a lighter, pinkish cytoplasm. The cell is surrounded by several red blood cells, which appear as smaller, orange-red discs.	Unknown
Mast cell	 A microscopic image of a mast cell, showing a large, round cell with a dark purple, granular nucleus and a lighter, pinkish cytoplasm. The cell is surrounded by several red blood cells, which appear as smaller, orange-red discs.	Release of granules containing histamine and other active agents

2.2. Cells of the immune system

Macrophages are phagocytes derived from blood monocytes

Monocyte: small, spherical cell with few projections, abundant cytoplasm, little endoplasmic reticulum, and many granules.

Migration of monocytes from the blood to various tissues:

- Kupffer cells, in the liver
- Alveolar macrophages, in the lung
- Splenic macrophages, in the red pulp
- Peritoneal macrophages, free-floating in peritoneal fluid
- Microglial cells, in the central nervous tissue

2.2. Cells of the immune system

Functions of Macrophages:

- A major function is to ingest microbes
- formation of cytoplasmic membrane-bound organelles
- fusion of these organelles with lysosomes,
- the enzymatic generation of reactive oxygen and nitrogen species in the lysosome that are toxic to microbes, and the digestion of microbial proteins by proteolytic enzymes.
- Macrophages ingest necrotic host cells (cleaning-up process after infection).

Efferocytosis: clearance of apoptotic cells by macrophage

- Macrophage phagocytosis of healthy host

Cells phagocytosis is prevented in part by an inhibitory receptor on the macrophage called (Signal regulatory protein α) SIRP α , which recognizes CD47 ("don't eat me" signal), a membrane protein on healthy cells.

2.2. Cells of the immune system

Cell-Adhesion Molecules

- The vascular endothelium regulate the movement of
 - blood-borne molecules and
 - leukocytes into the tissues.
- In order for circulating leukocytes to enter inflamed tissue or peripheral lymphoid organs, the cells must adhere to and pass between the endothelial cells lining the walls of blood vessels, a process called **extravasation**.
- Endothelial cells express leukocyte-specific **cell adhesion molecules (CAM)**

2.2. Cells of the immune system

Cell-Adhesion Molecules

- Leukocytes bear receptors that bind to CAMs on the vascular endothelium,
 - enabling these cells to extravasate into the tissues.

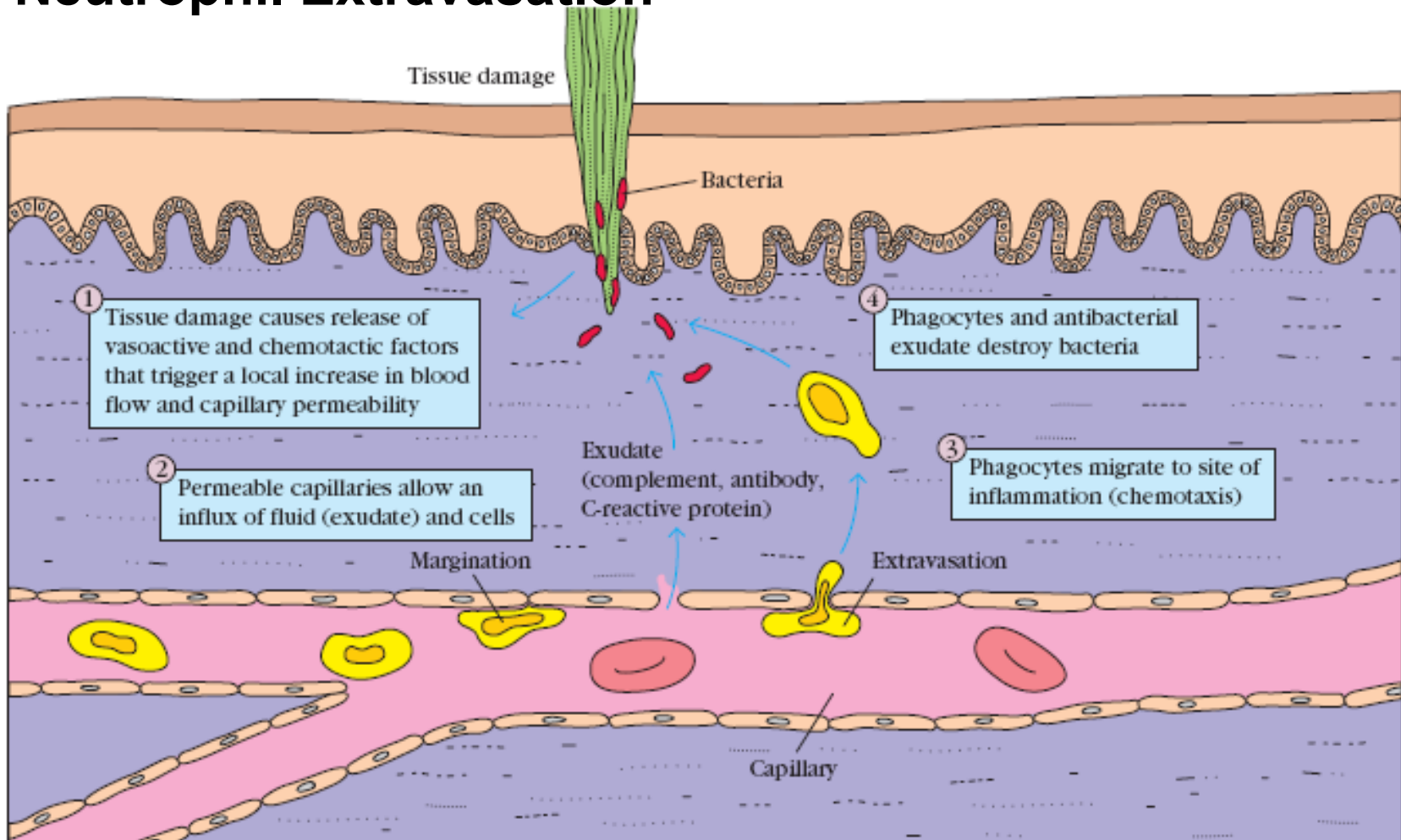
2.2. Cells of the immune system

Neutrophil Extravasation

- Neutrophils first bind to inflamed endothelium and extravasate into the tissues.
- Neutrophils recognize the inflamed endothelium and adhere
 - so that they are not swept away by the flowing blood.
- The bound neutrophils then penetrate the endothelial layer and migrate into the underlying tissue.
- Monocytes and eosinophils extravasate by a similar process,

2.2. Cells of the immune system

Neutrophil Extravasation



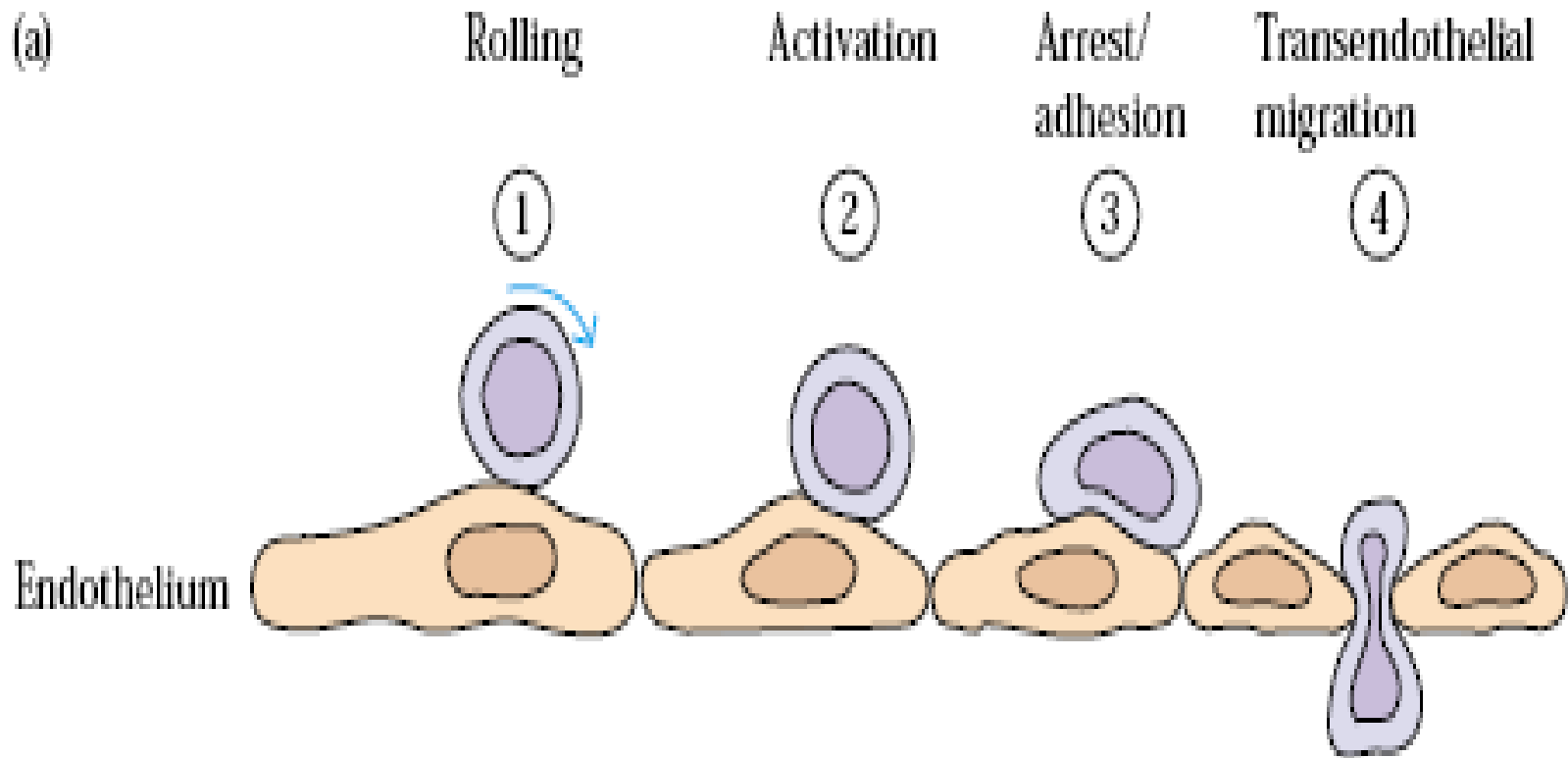
2.2. Cells of the immune system

Neutrophil Extravasation

- Neutrophil extravasation has four sequential steps:
 - Rolling,
 - Activation by chemoattractant stimulus,
 - Arrest and adhesion, and
 - Transendothelial migration.

2.2. Cells of the immune system

Neutrophil Extravasation



Extravasation of neutrophils

- **Step 1: Rolling**
- Phagocytes attach loosely to the endothelium by low-affinity, selectin-carbohydrate interactions. **E-selectin molecules** on the endothelium bind to **adhesion molecules** on the phagocyte membrane and bind the cell briefly, but the force of blood flow into the area causes the cell to detach and reattach repeatedly, rolling along the endothelial surface until stronger binding forces can be elicited.

Extravasation of neutrophils

- **Step 2: Activation by chemoattractant**
- Chemokines released in the area during inflammation, such as **interleukin 8 (IL-8)**, bind to receptors on the phagocyte surface and trigger an activating signal. This signal induces a conformational change in integrin molecules in the phagocyte membrane that **increases their affinity** for adhesion molecules on the endothelium.

Extravasation of neutrophils

- **Step 3: Arrest and adhesion**
- Interaction between integrins and adhesion molecules mediates the tight binding of the phagocyte to the endothelial cell.
- These integrin- adhesion molecules interactions also mediate the tight binding of phagocytes and their movement through the extracellular matrix.
- **Step 4: Trans endothelial migration**
- The phagocyte extends pseudopodia through the vessel wall and extravasates into the tissues.

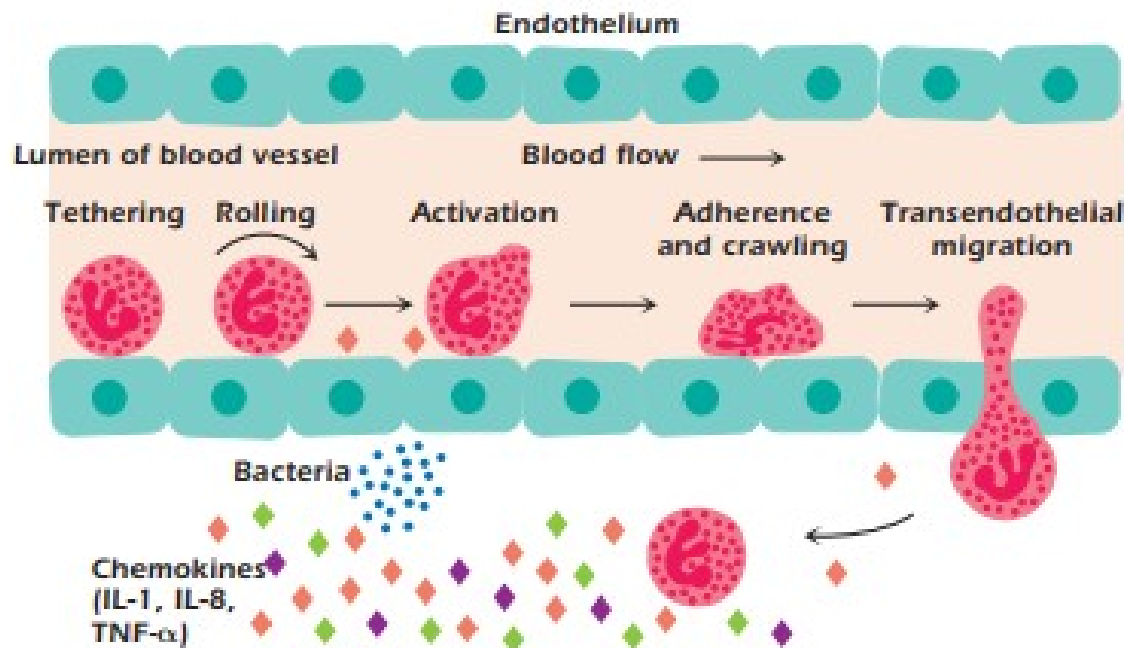


Figure 2.11. Leukocyte adhesion to endothelium leads to their adhesion, activation, and extravasation from the blood to tissue where they are needed to help destroy (e.g., phagocytize) pathogens such as bacteria that initiate this response.

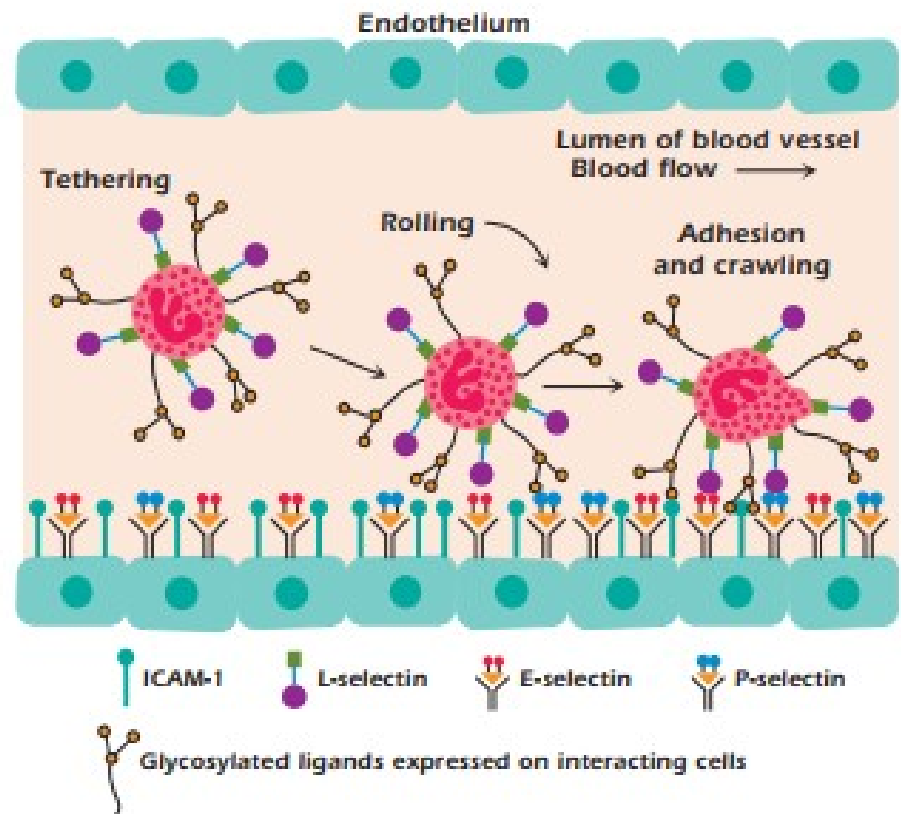


Figure 2.12. Adhesion molecules involved in leukocyte tethering, rolling, and adhesion to endothelium leading to transendothelial migration from blood to tissue.

2.2. Cells of the immune system

The Inflammatory Process

- Is a physiologic response to a variety of stimuli
 - Such as infections and tissue injury.

- An acute inflammatory response
 - Has a rapid onset
 - Lasts a short and
 - Accompanied by a systemic reaction known as the acute-phase response, which is characterized by a rapid alteration in the levels of several plasma proteins.

2.2. Cells of the immune system

The Inflammatory Process

- Chronic inflammation results in,
 - Persistent immune activation
 - Lead to formation of a **granuloma**
- The accumulation and activation of macrophages is the hallmark of chronic inflammation.

2.2. Cells of the immune system

The Inflammatory Process

- Two cytokines in particular, IFN- γ and TNF- α , play a central role in the development of chronic inflammation.
- TH1 cells, NK cells, and TC cells release IFN- γ , while activated macrophages secrete TNF- α .

2.2. Cells of the immune system

The Inflammatory Process

- Inflammatory Responses May Be Localized or Systemic
- The hallmarks of a localized acute inflammatory response, are
 - swelling (*tumor*)
 - redness(*rubor*)
 - heat (*calor*)
 - pain (*dolor*), and loss of function

2.2. Cells of the immune system

The Inflammatory Process

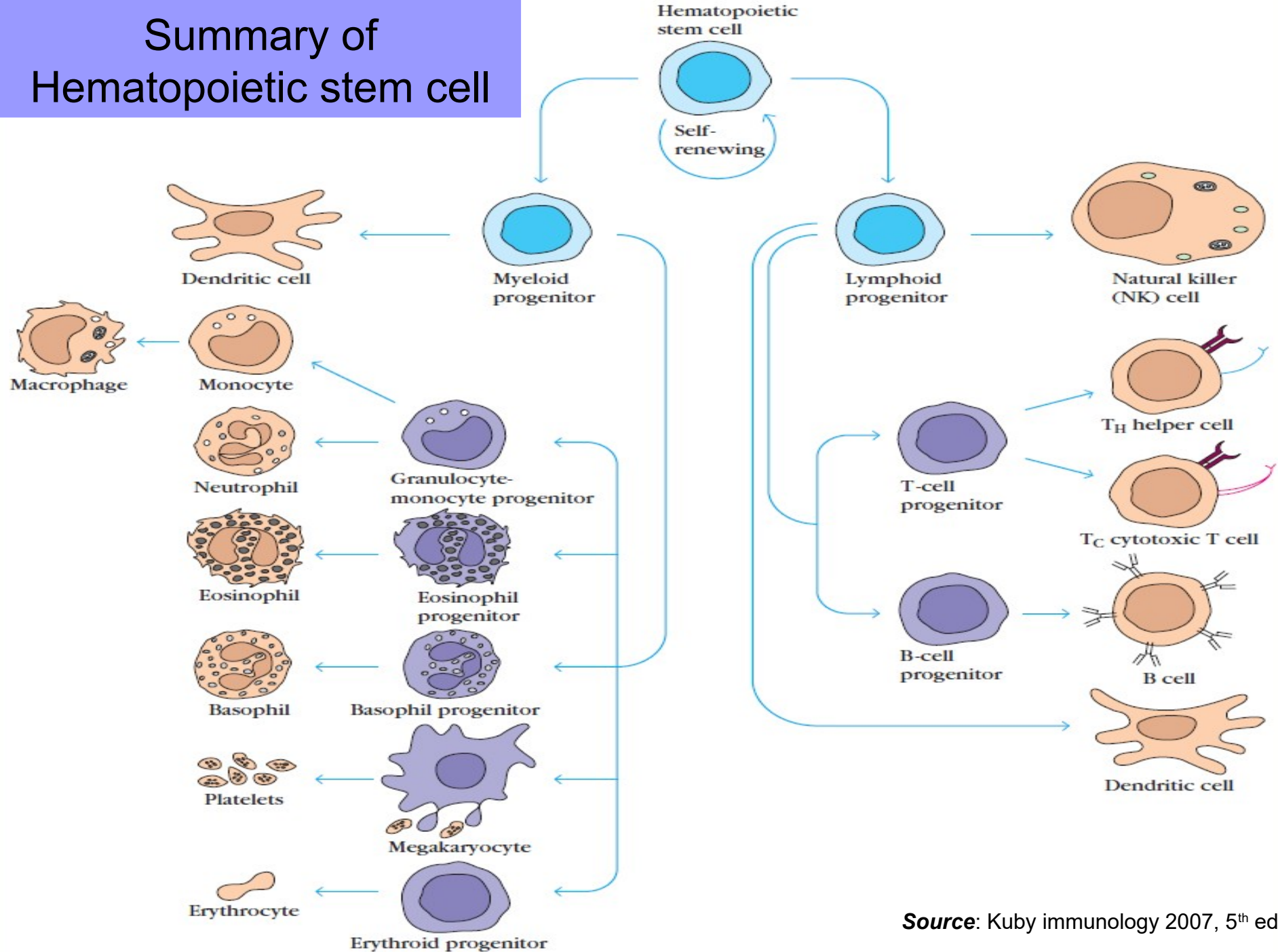
- The local inflammatory response is accompanied by a systemic response known as the **acute-phase response** , marked by
 - the induction of fever,
 - increased production of white blood cell (leukocytosis),
 - production of a large number of **acute-phase proteins** (C-reactive protein) in the liver.
 - Many systemic acute-phase effects are due to the combined action of IL-1, TNF- and IL-6.

Summary of Cells and organs of the immune system

Organs of the immune system

- Primary lymphoid organs
 - The primary lymphoid organs provide appropriate microenvironments for the development and maturation of lymphocytes. This includes Thymus, Bone marrow, Fetal liver

Summary of Hematopoietic stem cell



Source: Kuby immunology 2007, 5th ed

Summary of Cells and organs of the immune system

The secondary lymphoid organs trap antigen from defined tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with that antigen.

These are,

- Lymph nodes,
- Spleen,
- Mucosa Associated Lymph tissue (MALT),
- Tonsils, Payers patches, lamina propria (largest amount of lymphs), appendix collectively known Gut associated lymphoid tissue (GALT)
- Bronchial associated lymphoid tissue (BALT)

Antigens and immunogens

Malak Alshaikhali

MD, Al-Quds University

MSc Integrated Immunology, University Of Oxford.

Learning outcomes

- Understand the concept of immunogens, antigens, haptens, epitopes, adjuvants.
- Understand requirements of immunogenicity.
- Understand the nature of antigen recognition by B & T cells.
- Understand cross reactivity.

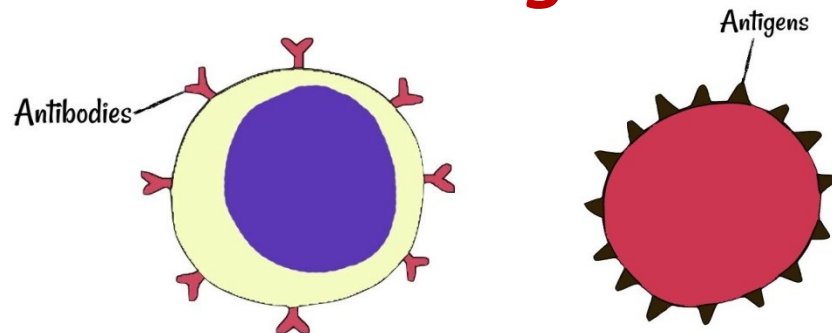
Immunogen vs. antigen

- **Definitions**

-Immunogen: any substance that can elicit an immune response.

-Antigen: any substance that can bind to a specific antibody.

All immunogens are antigens but not all antigens are immunogens.



Immunogen vs. antigen

- Hypothetically, any structure can be recognized by antibodies as an antigen.
- However, only protein antigens adaptive immune responses.
- Molecules such as carbohydrates, lipids and nucleic acids are all potential antigens, but will often only induce immune response if attached to a protein carrier.

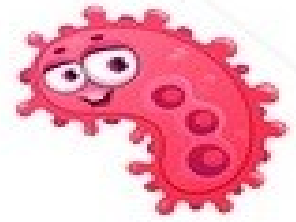
Immunogen vs. antigen

- Thus, the immunogenicity of protein antigens determines the outcomes of virtually every immune response.

Requirements for immunogenicity

- Foreignness.
- High molecular weight.
- Chemical complexity.
- Degradability.
- Other factors: genotype, lymphocytes repertoire, antigen dose, administration route.

Foreignness



- The first requirement for a compound to be immunogenic is foreignness.
- The more foreign the substance, the more immunogenic it is.
- Our immune system does not normally respond to self-constituents.
- *Autoimmune diseases* develop when tolerance to self-antigens is broken.

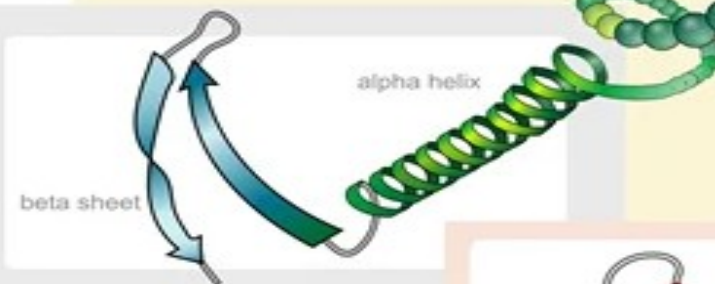
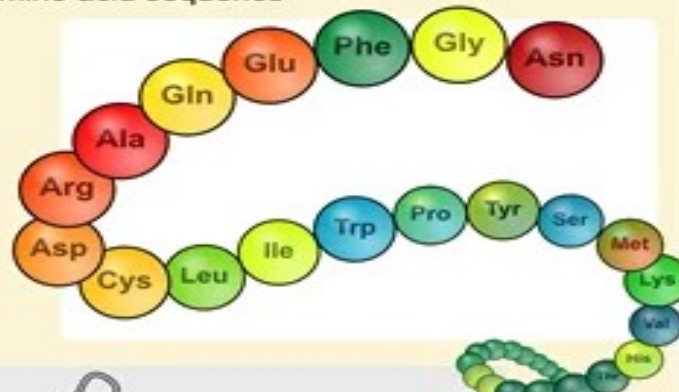
High Molecular Weight

- Poor immunogens: small compounds of molecular weight $<1,000$ Da.
e.g. penicillin, progesterone, aspirin
- Good immunogens: compounds of molecular weight >6000 Da.
e.g. albumin, tetanus toxin
- Rule of thumb: small substances have decreased immunogenicity and large substances have increased immunogenicity.

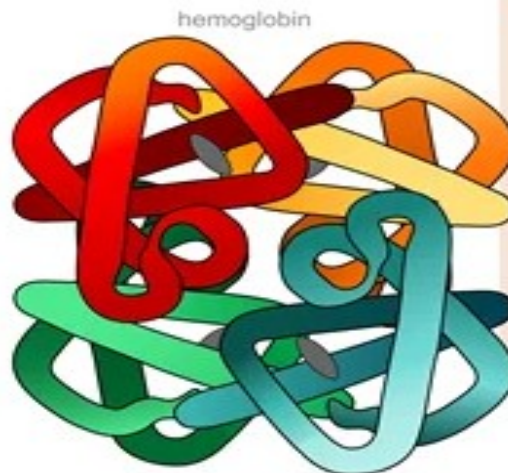
Chemical Complexity

- Physiochemically simple molecules are poorly immunogenic.
e.g. homopolymers of amino acid (polylysine) vs copolymers of several amino acids (poly-GAT)
- Increasing the complexity of these molecules by adding various moieties renders them immunogenic.
- The four levels of protein structure contributes to the molecule immunogenicity.

Primary structure
amino acid sequence



Secondary structure
regular sub-structures



Quaternary structure
complex of protein molecules



Tertiary structure
three-dimensional structure

Degradability

- T cell recognize antigenic epitopes presented by MHC molecules on APC.
- Antigen processing: APC degrade antigens enzymatically to produce epitopes (a.k.a antigenic determinant).
- Degraded antigens binds noncovlantly to MHC molecules on APC which stimulate the activation and clonal expansion of antigen-specific effector T cells.

Degradability cont.

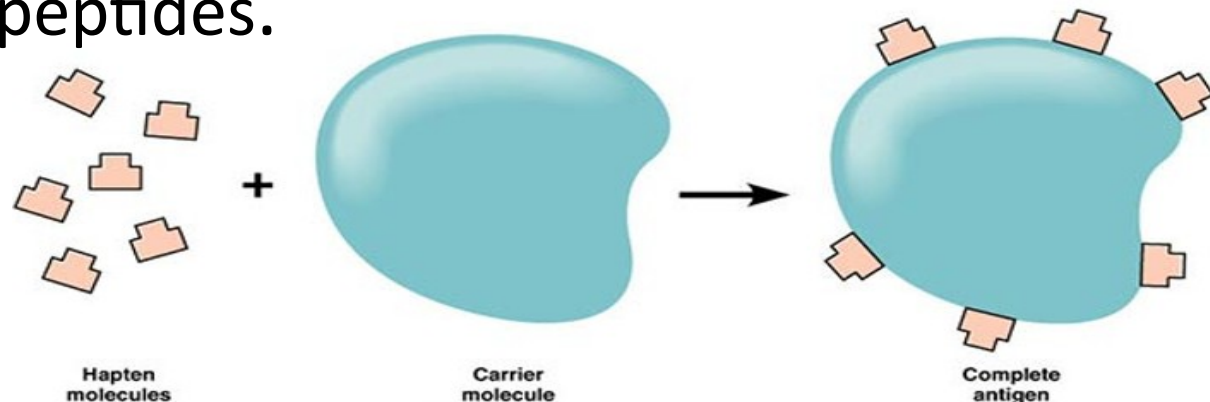
- A protein antigen's susceptibility to enzymatic degradation depends on:
 1. Protein structural stability.
 2. Protein susceptibility to partial enzymatic digestion e.g. D-amino acids vs L-amino acids.
- Carbohydrates are not processed or presented and are thus unable to activate T cells, although they can directly activate B cells

Factors that influence the immunogenicity of proteins

Parameter	Increased immunogenicity	Decreased immunogenicity
Size	Large Small (MW<2500)	
Dose	Intermediate	High or low
Route	Subcutaneous > intraperitoneal > intravenous or intragastric	
Composition	Complex	Simple
Form	Particulate	Soluble
	Denatured	Native
Similarity to self protein	Multiple differences Few differences	
Adjuvants	Slow release Rapid release	
	Bacteria	No bacteria
Interaction with host MHC	Effective	Ineffective

Haptens

- **Definition:** *(from the Greek haptain, to fasten/to grasp)*
Small organic molecules of simple structure, that do not provoke immune response when injected by themselves. However, antibodies can be raised against them if the molecule is attached covalently to a protein *carrier*.
- **Examples:** drugs, antibiotics, cosmetics, food additives, small peptides.



Other requirements for immunogenicity

- Antigen dose: a threshold dose of antigen must be given to elicit adequate immune response.
 - Doses below threshold: no response
 - Doses above threshold: increase in response until plateau.
 - Very high doses: decline in immune response/ development of tolerance e.g. to abundant self-antigens.

Other requirements for immunogenicity

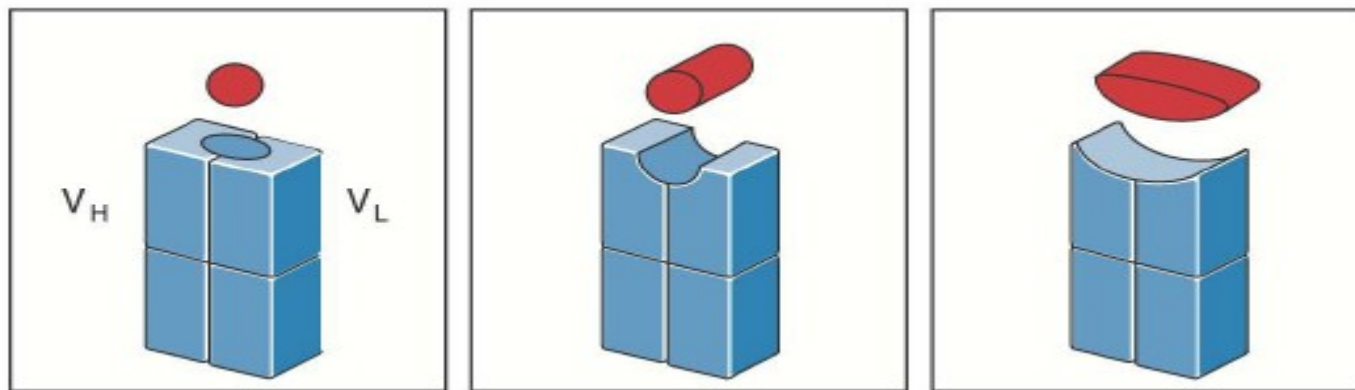
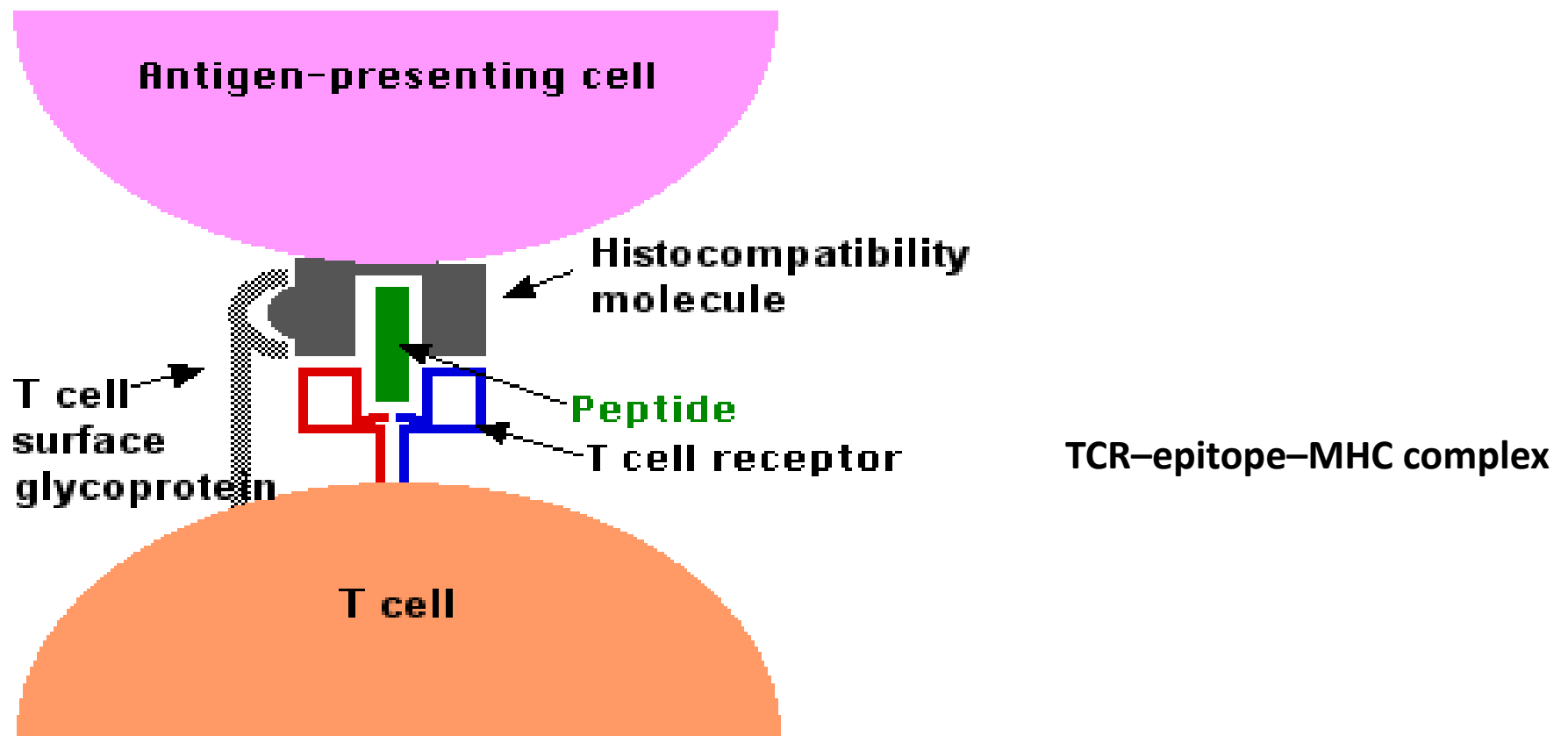
- Route of administration
 - Subcutaneous route elicits the strongest response: role for Langerhans cells in the skin.
 - Systemic: induce immune unresponsiveness or tolerance if antigens did not form aggregates or binds host cells.
 - Oral: induce local antibody response in the gut and systemic tolerance.

What is immune response

- It is induced by an antigen which generates antibodies or lymphocytes that react specifically with the antigen.
- Lymphocytes receptors have binding sites for antigens with special structural characteristics.
- For example, antigenic determinants/epitopes binds part of the immunoglobulin in the called complementarity-determining region (CDR)

What is immune response cont.

- Epitopes that binds B-cell receptors/antibodies are usually 5-7 amino acids long.
- T-cells binds larger epitopes, usually 8-12 amino acids long.
- They bind epitopes bound non-covalently to MHC molecules on APC.
- This bimolecular epitope–MHC complex then binds to the TCR, forming a trimolecular complex (TCR–epitope–MHC)



Antigens can bind in pockets, grooves, or on extended surfaces in the binding sites of antibodies.

Types of immune response

- Primary response
occurs after the first exposure to an immunogen.
- Events that occur after the initial immunization:
 - APC cells process antigen, triggering antigen-specific lymphocytes to proliferate and differentiate.
 - T-lymphocyte subsets interact with other subsets and induce the latter to differentiate into T lymphocytes with specialized function e.g. memory cells.
 - T lymphocytes also interact with B lymphocytes, inducing them to synthesize and secrete antibodies.

Types of immune responses

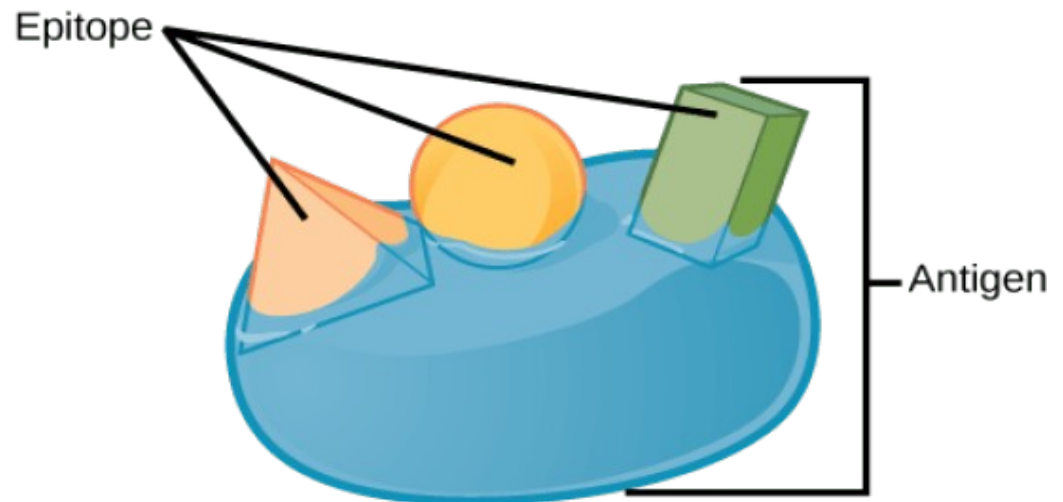
- Secondary response

Occurs after the second exposure to an immunogen when the primary response has subsided.

- Events that occur after repeated exposure:
 - The response is quicker onset and stronger.
 - Uses antigen specific lymphocytes generated in the primary response- memory cells.
 - It acts as if the body remembered the previous event.

Epitopes

- Epitopes are also known as ***antigenic determinants***. They are the part of an antigen that is recognized by the immune system and are the smallest unit of antigen that is capable of binding with antibodies and T-cell receptors.



Epitopes recognized by B-and T-cells

Characteristic	B-cells	T-cells
Antigen interaction	BCR-Ag/ Ab-Ag	TCR-Ag-MHC
Nature of antigens	Proteins, lipids, polysaccharide	Only proteins
Binding soluble antigens	Yes hydrophilic	No hydrophobic
Epitopes recognized	On the outside of antigen, can be conformational, discontinuous.	Internal linear/continuous peptides produced by antigen processing (proteolytic degradation)
Example	Terminal side chains of polysaccharides , hydrophilic portions on protein molecule.	

Major classes of antigens

- Polysaccharides.
 - Only immunogenic when associated with protein carriers. As of glycoproteins.
 - They induce antibody-mediated immune response
 - For example antibodies against polysaccharide molecules in microorganisms and eukaryotic cells
 - Examples: antibodies produced against ABO blood groups which are polysaccharides on RBC surface.

Major classes of antigens

- Lipids
 - Only immunogenic if attached to carrier protein.
 - By definition, may be regarded as haptens!
- Nucleic acids
 - Only immunogenic when conjugated to proteins.
 - Examples: anti-DNA antibodies detected in patients with SLE (systemic lupus erythematosus).

Major classes of antigens

- Proteins
 - The main inducer for immune responses.
 - The greater the complexity of a protein the greater the immune response.
 - Proteins contains multiple epitopes that could be targeted by antigen-specific lymphocytes.

Forces affecting antigens binding to lymphocytes

- The binding of antigen with antibodies or TCRs involve non-covalent bonds only.
- These include electrostatic interactions, hydrophobic interactions, hydrogen bonds, and van der Waals forces.
- They are relatively weak forces, thus the surface of interaction between the Ag and the complementarity region on Ag-receptor needs to be large enough to allow the summation of all possible interactions.

Adjuvants

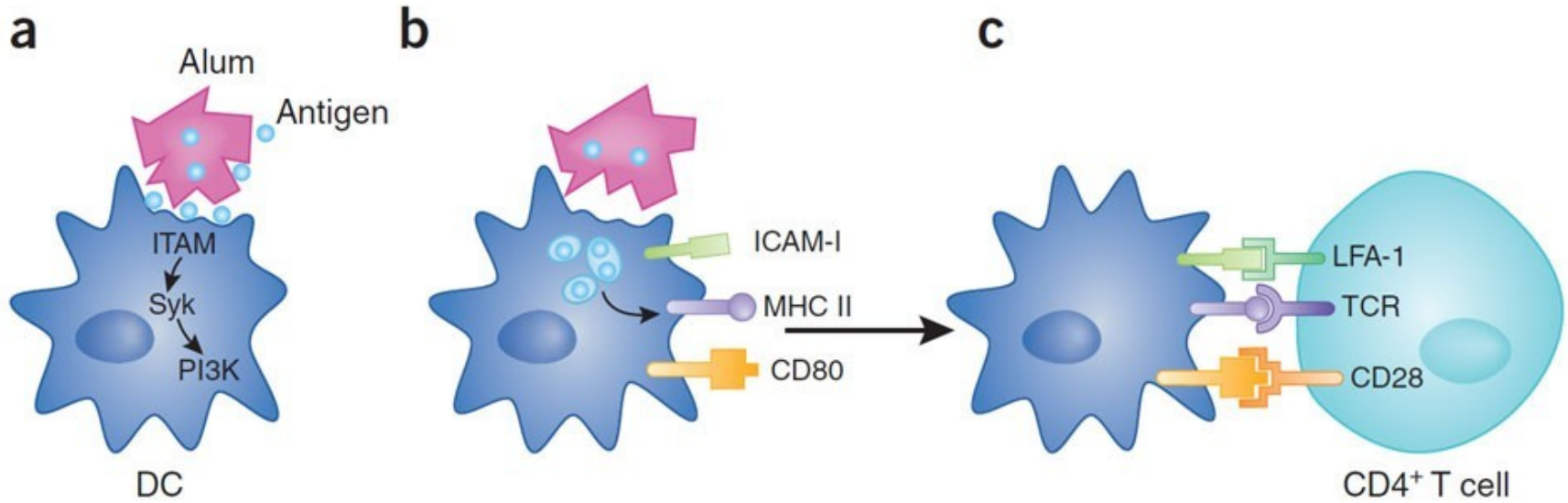
- Definition:

Any substance that enhances the immunogenicity of substances mixed with it.

- They are different from carrier proteins that bind haptens.
- Adjuvants are mixed with the antigen and usually render it particulate, which helps to retain the antigen in the body and promotes uptake by macrophages.

Vaccine administration site

Draining lymph node



Adjuvant vs. Carrier protein

Characteristics	adjuvant	Carrier protein
Type of bonding to immunogen	Weak linkage	Stable linkage (usually covalent)
Role in eliciting immune response	Needed for initial immunization to elicit primary response	Required to illicit initial and subsequent responses to haptens
Effects on immunogenicity	Renders immunogens more immunogenic	A hapten will not become immunogenic when mixed with adjuvant.

Mechanisms adjuvants enhance immunogenicity

- Adjuvants improve antigen delivery and antigen processing and presentation by APCs as follows:
 1. They convert soluble protein antigens into particulate material, which is more readily ingested by antigen-presenting cells such as macrophages.

Mechanisms adjuvants enhance immunogenicity cont.

2. Adjuvants containing microbial products signals APC to become more effective by inducing the production of inflammatory cytokines and to express co-stimulatory molecules.
3. Increase the biological or immunological half-life of vaccine antigens.

Adjuvants that enhance immune responses

Adjuvant name	Composition	Mechanism of action
Incomplete Freund's adjuvant	Oil-in-water emulsion	Delayed release of antigen; enhanced uptake by macrophages
Complete Freund's adjuvant	Oil-in-water emulsion with dead mycobacteria	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators in macrophages
Freund's adjuvant with MDP	Oil-in-water emulsion with muramyl dipeptide (MDP), a constituent of mycobacteria	Similar to complete Freund's adjuvant
Alum (aluminum hydroxide)	Aluminum hydroxide gel	Delayed release of antigen; enhanced macrophage uptake
Alum plus <i>Bordetella pertussis</i>	Aluminum hydroxide gel with killed <i>B. pertussis</i>	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators
Immune stimulatory complexes (ISCOMs)	Matrix of Quil A containing viral proteins	Delivers antigen to cytosol; allows induction of cytotoxic T cells

Use of adjuvants in vaccines

- Adjuvants are used in peptide-based vaccines and vaccines based on highly purified protein components.
- Peptide vaccines do not activate the innate immune system in the same way as a natural infection and require additional components a.k.a adjuvants to mimic how infections normally activate innate immunity, which induces dendritic cells to become optimally stimulatory for T cells.

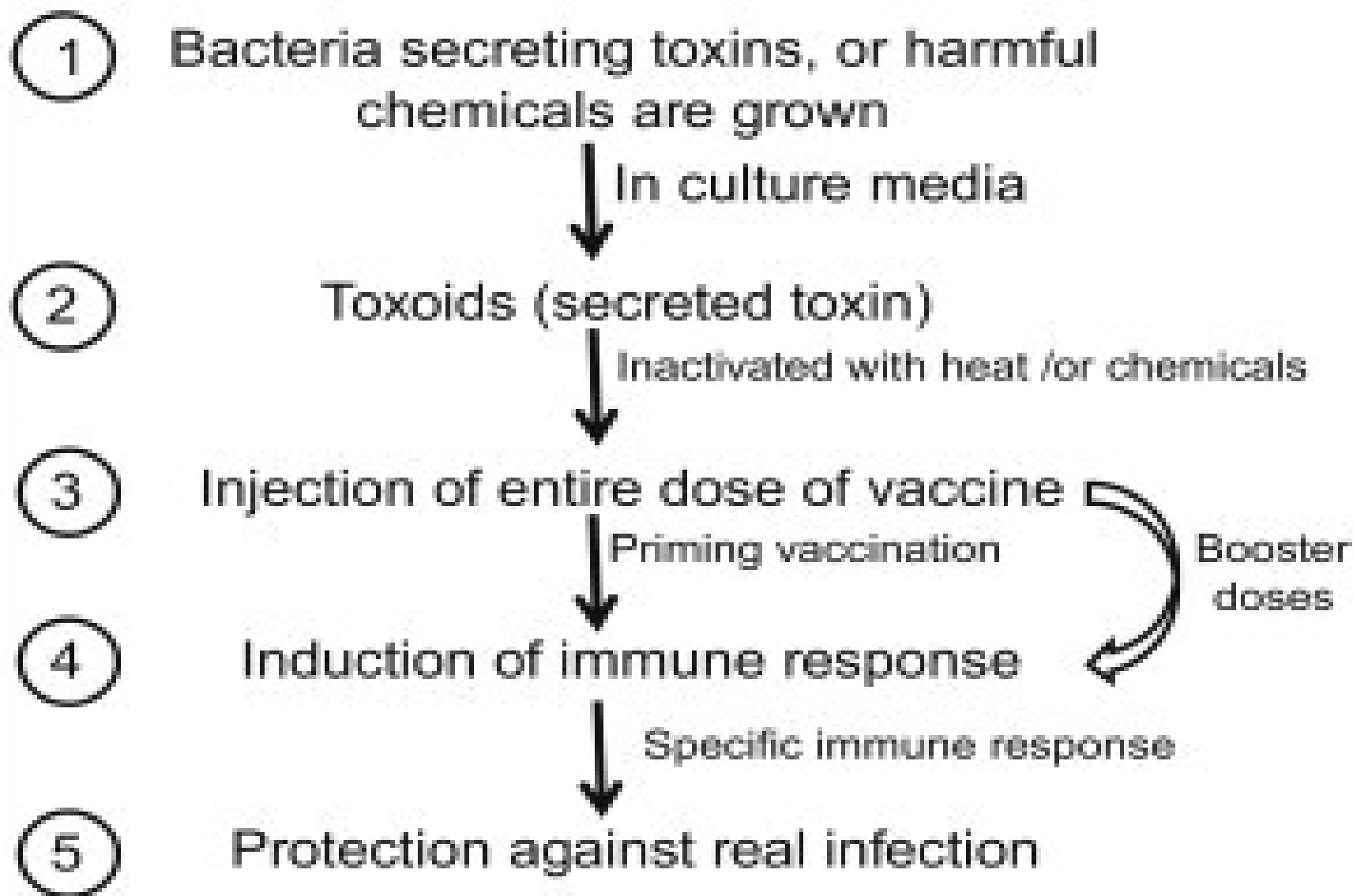
What is a toxoid?

- Large macromolecular antigens contains many potential epitopes.
- This provides a chance to biologically manipulate their structure/harmful characteristics without altering their immunogenicity.
- This concept comes in use when immunizing against highly pathogenic organisms or their toxic products.

What is a toxoid? cont.

- For example, *tetanus toxoid* is a tetanus toxin that has been modified chemically or genetically by removing its toxicity while maintaining its immunochemical characteristics.
- The toxoid is then used to immunize individuals to protect against tetanus infection.
- Thus we can say that a humoral immune response to a toxoid ***cross-reacts*** immunologically with the toxin.

Toxoids



Tetanus vaccine

- *Tetanus toxoid* is not immunogenic in the absence of adjuvants, so tetanus toxoid vaccines contain alum, which bind to the toxoid.

Pertussis vaccine

- Pertussis toxin and toxoid has adjuvant properties in itself.
- When given mixed as a toxoid with tetanus and diphtheria toxoids, not only protects against whooping cough but also acts as an additional adjuvant for the other two toxoids.
- This mixture makes up the DTP triple vaccine given to infants in the first year of life.

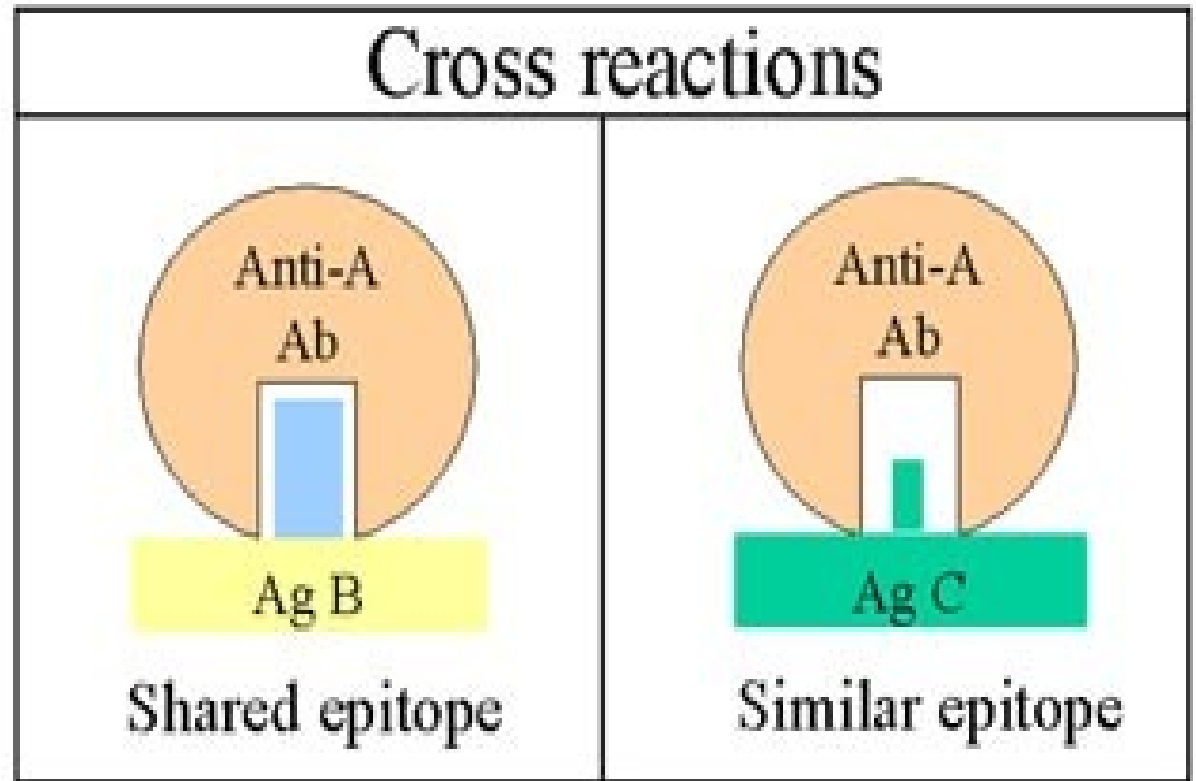
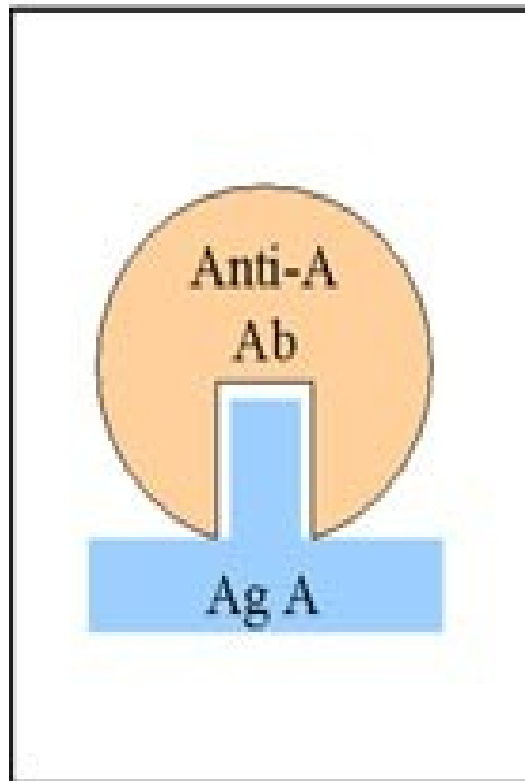
Cross reactivity

- Definition

The binding of an antibody or a T-cell to an antigen other than the immunogen.

- Cross reaction can occur between antigens that have identical epitopes or have structural resemblance.
- Most antibodies cross-react with closely related antigens but, on occasion, some bind antigens with no clear relationship to the immunogen.

Cross reactivity cont.



Cross reactivity cont.

- Example 1

(Immunologic cross-reactivity, wherein the two cross-reacting substances are related to each other)

Tetanus toxoid protects against tetanus Although the molecules of toxin and toxoid differ in many physicochemical and biologic respects, they cross-react immunologically. They share enough epitopes to allow the immune response to the toxoid to mount an effective defense against the toxin itself.

Cross reactivity cont.

- Example 2

(Immunologic cross-reactivity, wherein the two cross-reacting substances are unrelated to each other, a.k.a heterophile antigens)

- Cross-reactivity between foreign molecules on pathogens and self molecules can lead to anti-self responses and autoimmune disease.
- For example, human blood group A antigen reacts with antibodies raised against pneumococcal capsular polysaccharide (type XIV).
- Human blood group B antigen reacts with antibodies to certain strains of *Escherichia coli*.

Conjugate vaccines

- Many bacteria- e.g *Neisseria meningitidis*, *Streptococcus pneumoniae* , and *H. influenzae*- have an outer capsule composed of polysaccharides.
- The most effective defense against these microorganisms is opsonization of the polysaccharide coat with antibody.
- The aim of vaccination is therefore to elicit antibodies against the polysaccharide capsules of the bacteria.
- Capsular polysaccharides can be harvested from bacterial growth medium and, because they are T-cell independent antigens, they can be used on their own as vaccines.

Conjugate vaccines cont.

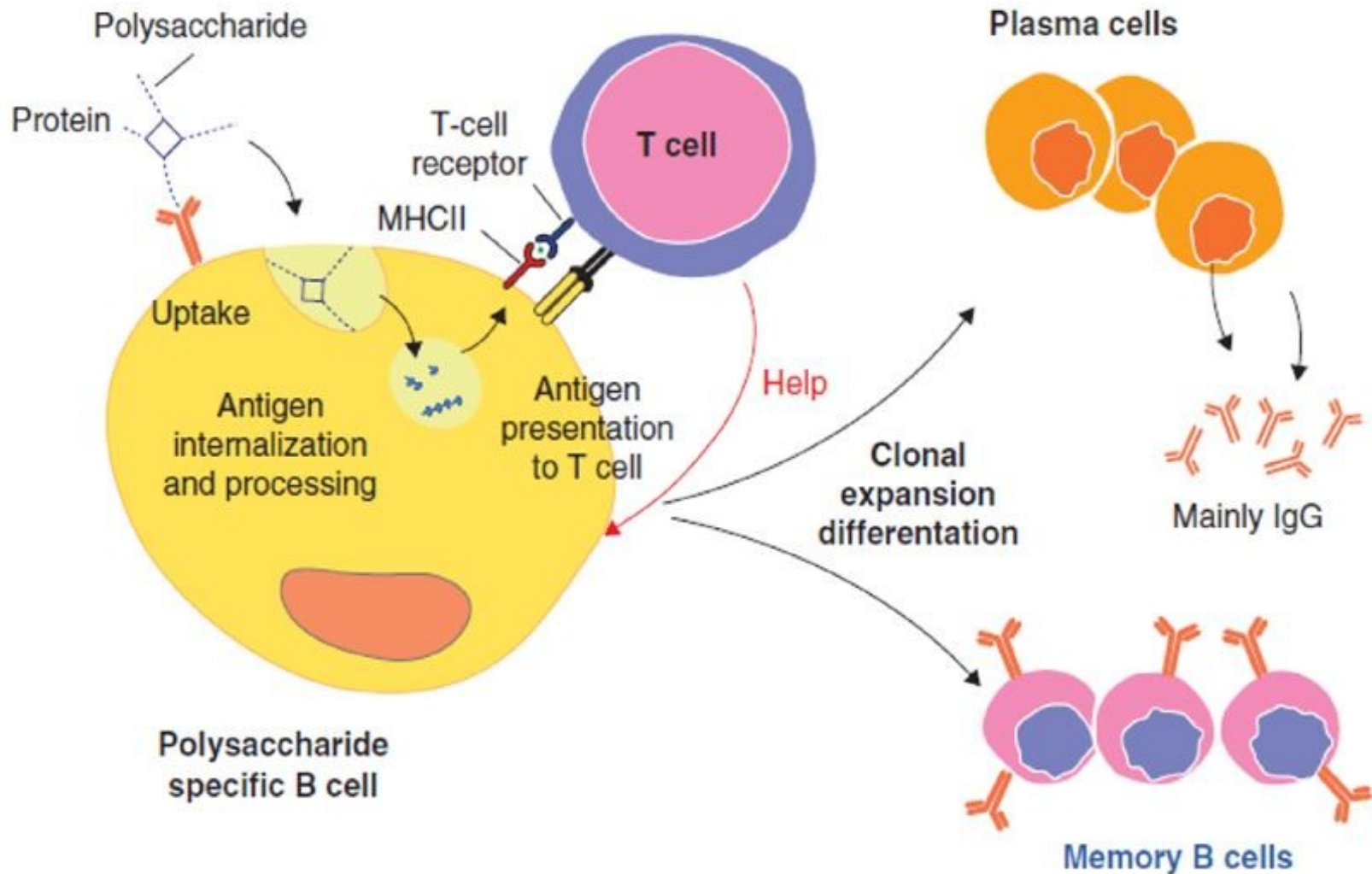
- **Problem-**

young children under the age of 2 years cannot make good T-cell independent antibody responses and cannot be vaccinated effectively with polysaccharide vaccines.

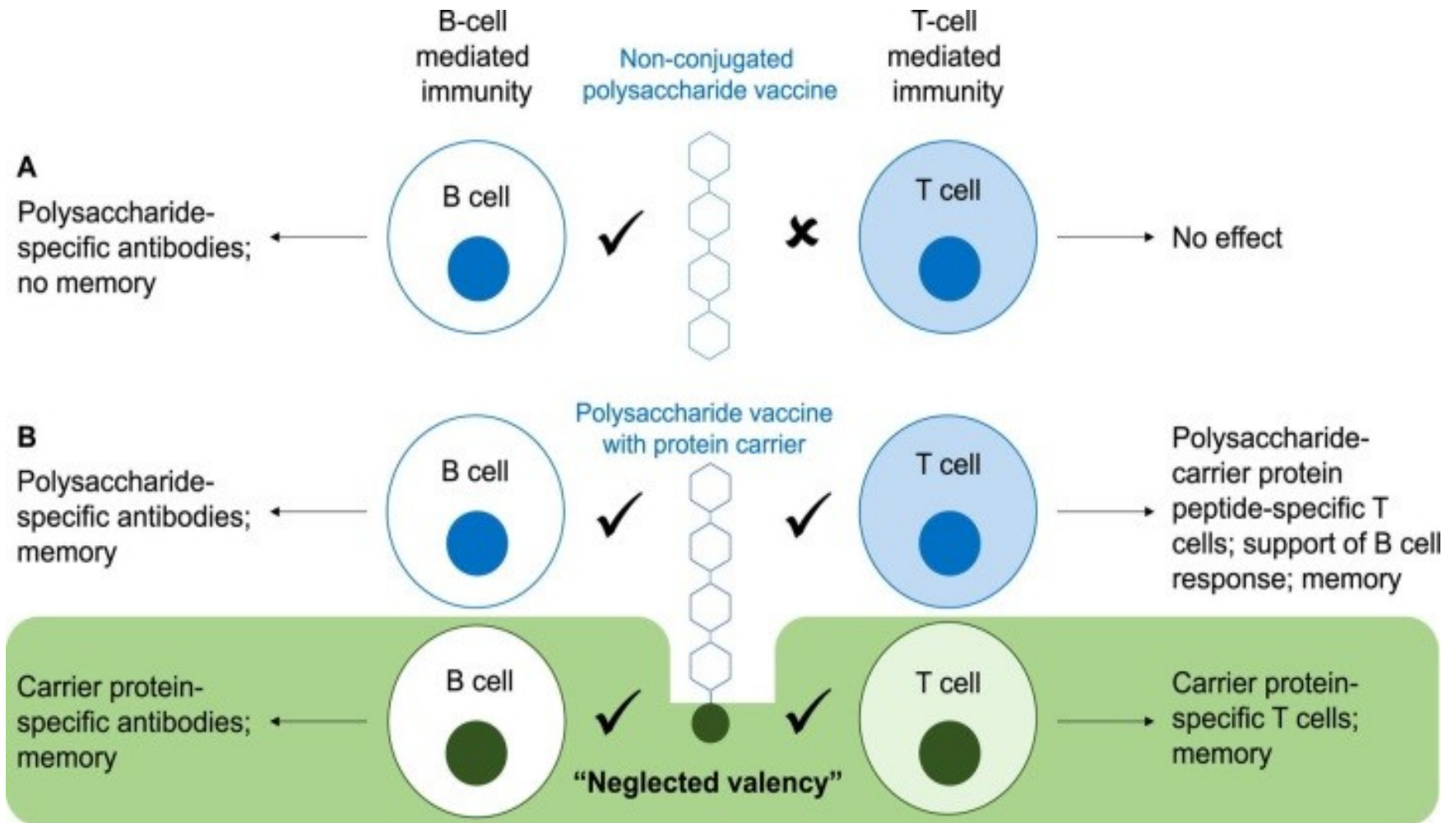
- **Solution-**

Bacterial polysaccharides are conjugated chemically to protein carriers, which provide peptides that can be recognized by antigen specific T cells, thus converting a T-cell independent response into a T-cell dependent antipolysaccharide antibody response.

Conjugate vaccines



Conjugate vaccines



Immunoglobulins

“Antibodies”

Dr. Dalia Wehedi

Introduction

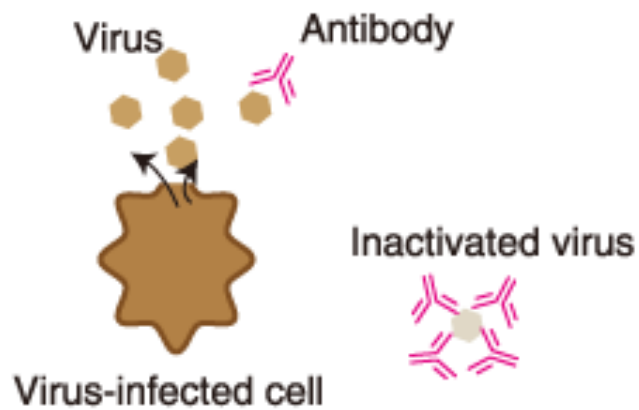
- Antibodies are globulin proteins (immunoglobulins) that are synthesized in serum and tissue fluids, which react specifically with the antigen that **stimulated their production**.
- Three types of globulins are present in the blood: alpha, beta, and gamma.
- The antibodies are the **gamma globulins**.
- Antibodies are one of the major plasma proteins, and against infection.
- The most important function of antibodies is to confer protection against microbial pathogens.

Introduction

Antibodies confer protection in the following ways:

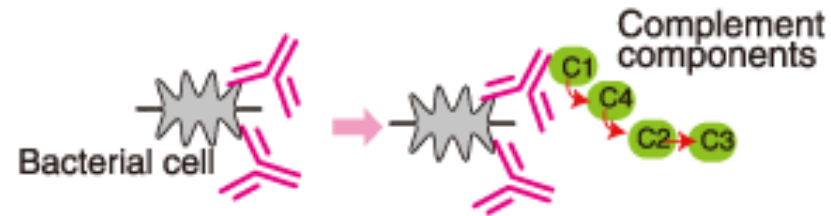
1. They prevent attachment of microbes to mucosal surfaces of the host.
2. They reduce virulence of microbes by neutralizing toxins and viruses.
3. They facilitate phagocytosis by opsonization of microbes.
4. They activate complement, leading to complement-mediated activities against microbes.

Neutralization



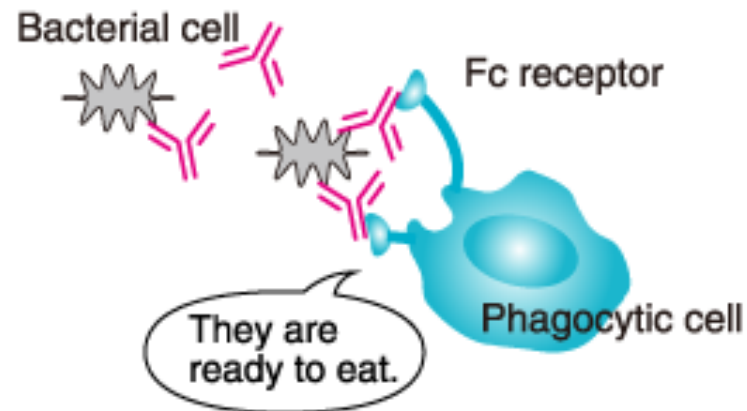
Antibodies bind to and inactivate viruses and toxins. These antibodies are called "neutralizing antibodies."

Complement recruitment by antibodies



Antigen-antibody complexes activate the complement system (the classical pathway), triggering its antibacterial activity.

Opsonization



Phagocytic cells grab the antibodies bound to the surface of foreign substances, for efficient phagocytosis.

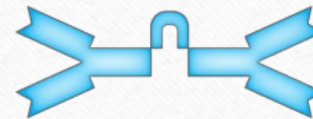
Immunoglobulins

- There are five classes of immunoglobulins:

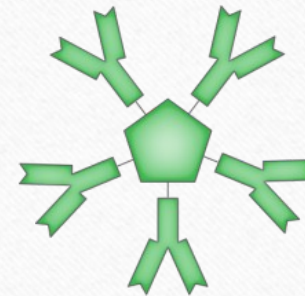
- (i) Immunoglobulin G (IgG)
- (ii) Immunoglobulin M (IgM)
- (iii) Immunoglobulin A (IgA)
- (iv) Immunoglobulin E (IgE)
- (v) Immunoglobulin D (IgD)



Monomer
IgD, IgE, IgG



Dimer
IgA



Pentamer
IgM

5 Types of Antibodies

Antibodies or immunoglobulins (Ig) are Y-shaped proteins that recognize unique markers (antigens) on pathogens.



IgA

Secreted into mucous, saliva, tears, colostrum. Tags pathogens for destruction.



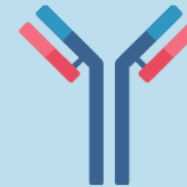
IgD

B-cell receptor. Stimulates release of IgM.



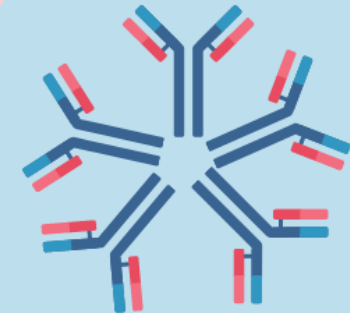
IgE

Binds to mast cells and basophils. Allergy and antiparasitic activity.



IgG

Binds to phagocytes. Main blood antibody for secondary responses. Crosses placenta.



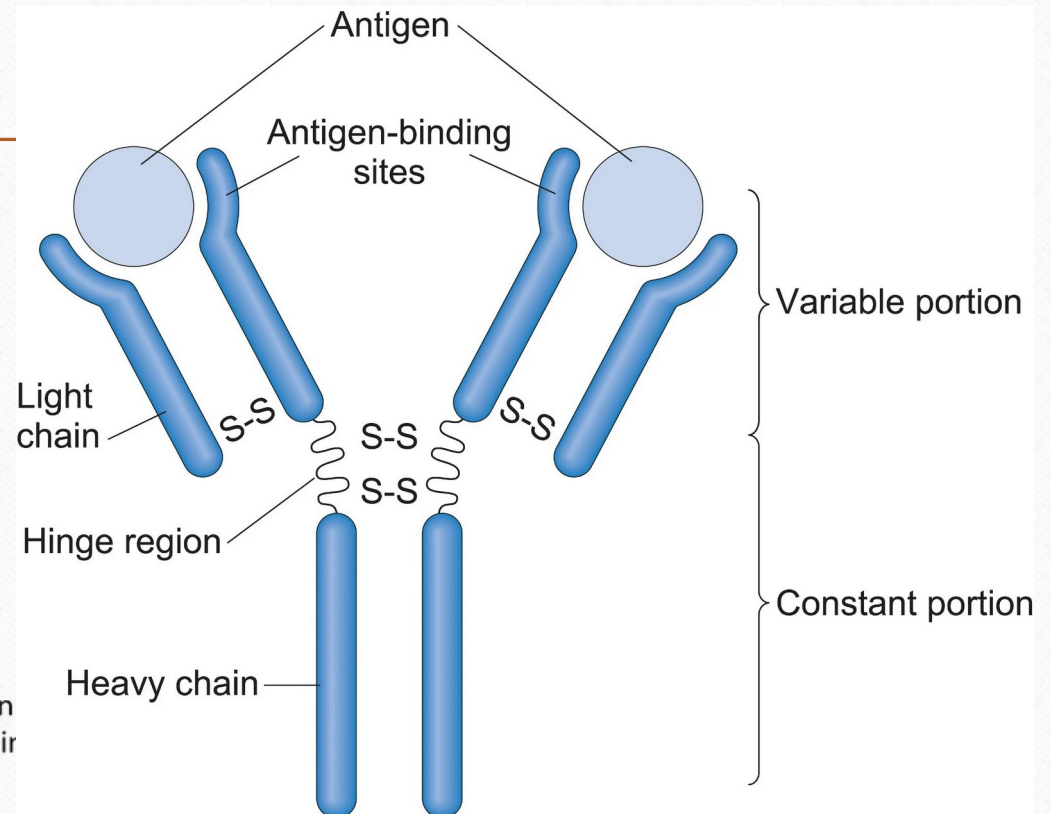
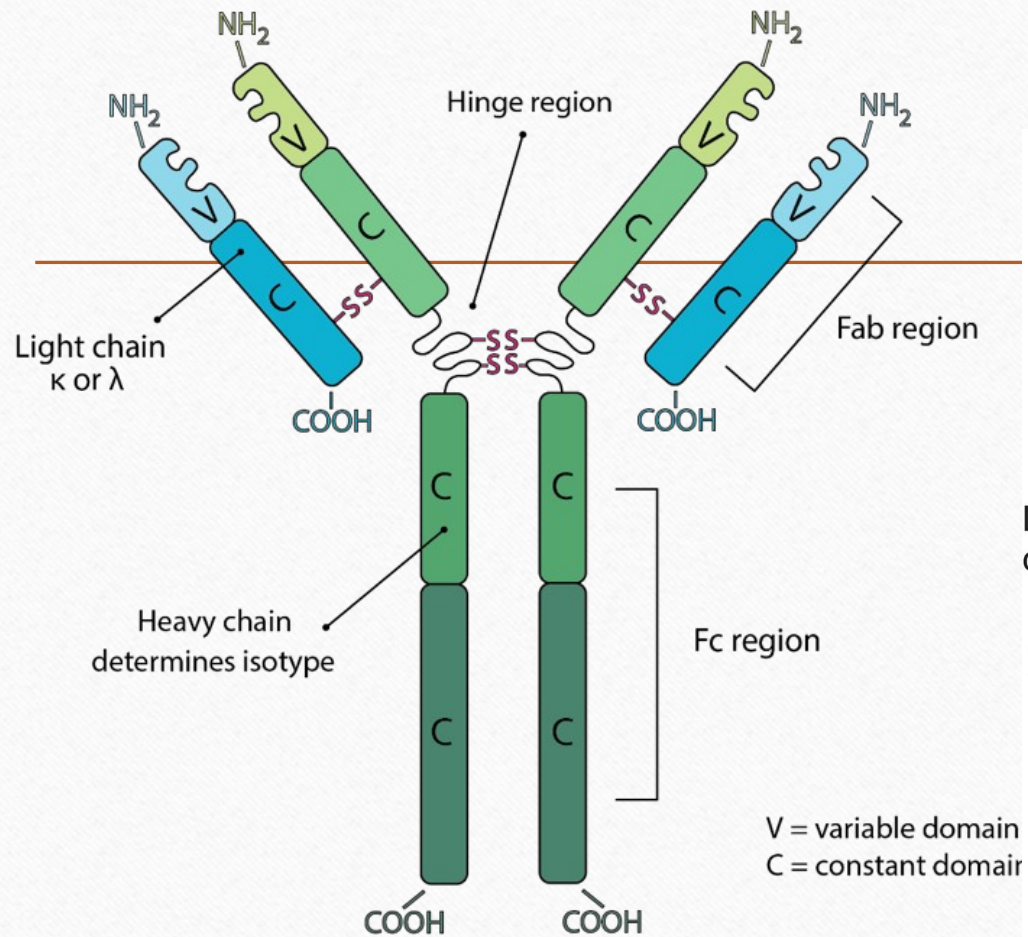
IgM

Fixes complement. Main antibody of primary responses. B-cell receptor. Immune system memory.

Structure of immunoglobulins

Immunoglobulins show the following properties:

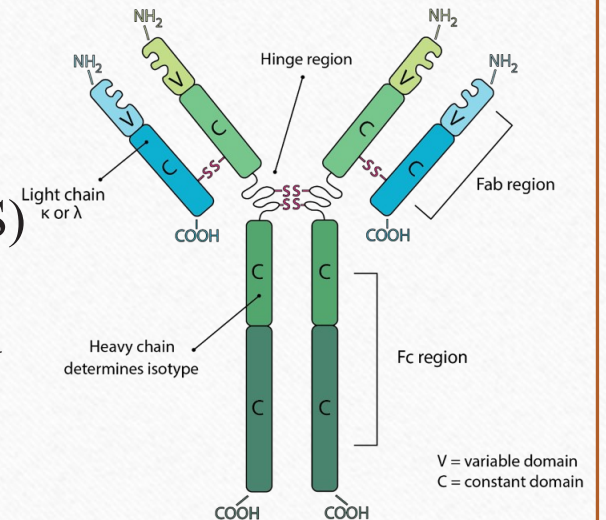
- They are glycoproteins.
- They are a complex structure of four polypeptide chains: two identical heavy chains and two identical light chains. This gives immunoglobulin an overall 'Y' or 'T' shape, which is the most widely recognized feature of immunoglobulin structure.
- The terms “heavy” and “light” refer to the molecular weights of the chains. The heavy chains are longer, and light chains are shorter.



Structure of immunoglobulins

Heavy chains:

- An immunoglobulin molecule has two heavy chains.
- Each heavy chain is made up of 420–440 amino acids.
- The two heavy chains are held together by one to five disulfide (S—S) bonds.
- Each heavy chain is bound to a light chain by a disulfide bond and by noncovalent bonds to form a heterodimer (H—L).



Structure of immunoglobulin

TABLE 13-1

Classes of immunoglobulins and their heavy chains and subclasses

Class	Heavy chain	Subclasses
IgG	Gamma	$\gamma_1, \gamma_2, \gamma_3, \gamma_4$
IgM	Mu	None
IgA	Alpha	α_1, α_2
IgE	Epsilon	None
IgD	Delta	None

Structure of immunoglobulins

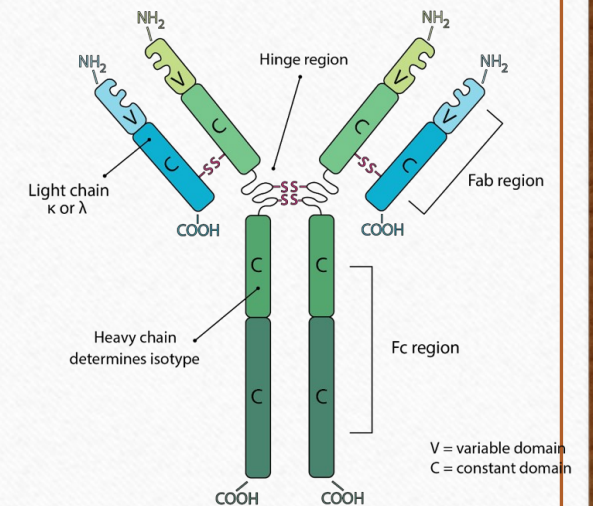
Light chains

- An immunoglobulin molecule has two light chains.
- Each light chain is made up of 220–240 amino acids.
- Light chain is attached to the heavy chain by a disulfide bond.
- The light chains are structurally and chemically similar in all classes of immunoglobulins.
- They are of two types: kappa (κ) and lambda (λ).
- Each immunoglobulin has either **two (κ) or two (λ) chains** but never both.
- The kappa and lambda chains are present in human serum in a ratio of 2:1

Structure of immunoglobulins

Variable and constant chains:

- Each polypeptide chain of an immunoglobulin molecule contains an amino terminal part and a carboxy terminal part.
- The amino terminal part is called the variable region (***V region***) and the carboxy terminal part is called the constant region (***C region***). Both heavy and light chains contain variable and constant regions.
- Each heavy chain consists of one variable (VH) and three constant (CH) domains.
- Each light chain consists of one variable (VL) and one constant domain (CL).



Structure of immunoglobulins

Variable region:

- The amino-terminal half of the light or heavy chain, consisting of 100–110 amino acids
- V region is different for each class of immunoglobulin.
- The variable regions of both light and heavy chains consist of three highly variable regions known as *hypervariable regions*.
- The antigen combining sites Fab of the antibody molecule that consists of only 5–10 amino acids each are present in the hypervariable region of both the light and heavy chains.
- The high specificity of antibodies is primarily due to the presence of these hypervariable regions

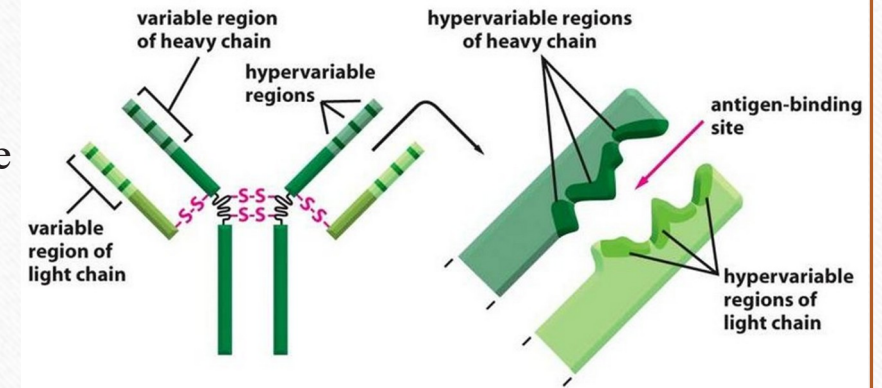


Figure 25-31 Molecular Biology of the Cell 5/e (© Garland Science 2008)

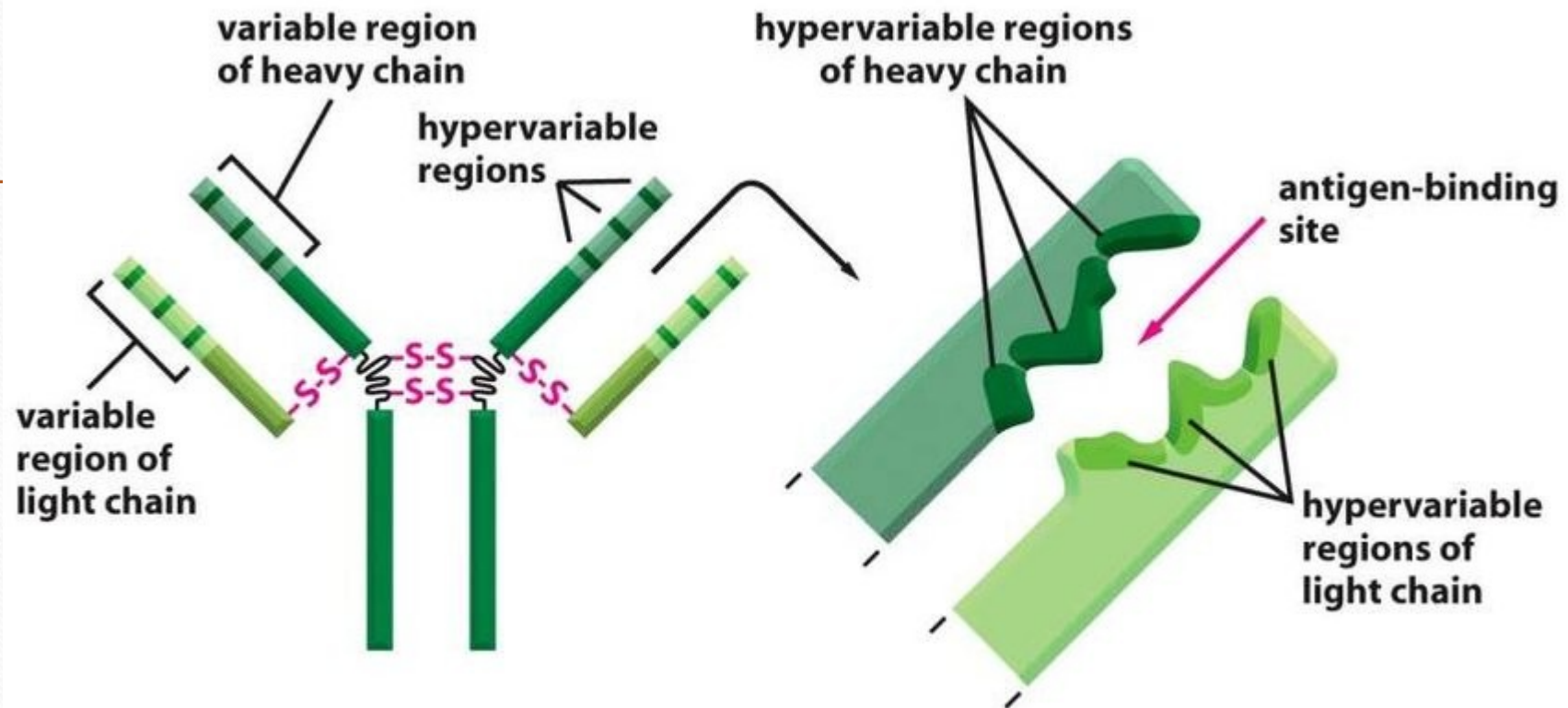


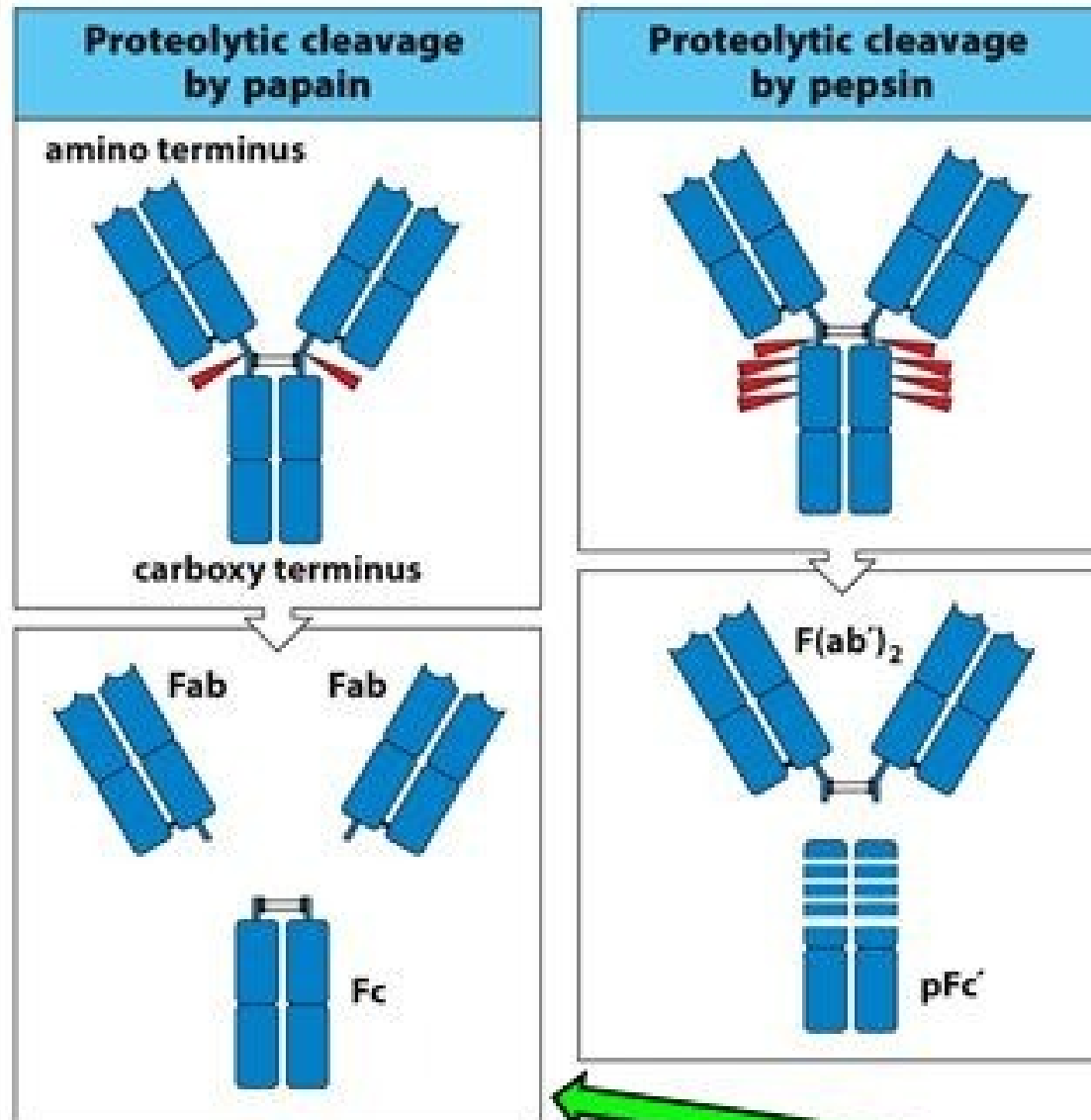
Figure 25-31 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Structure of immunoglobulins

Constant region:

- The carboxyl-terminal half of the molecule is called the constant (C) region.
- The constant region of the heavy chain has many biological functions.
- It is responsible for **activation of the complement, binding to cell surface receptors, placental transfer, and many other biological activities.**
- The constant region of the light chain has no biological function.
- A single antibody molecule has two identical heavy chains and two identical light chains, H₂L₂, or a multiple (H₂L₂)_n of this basic four-chain structure.

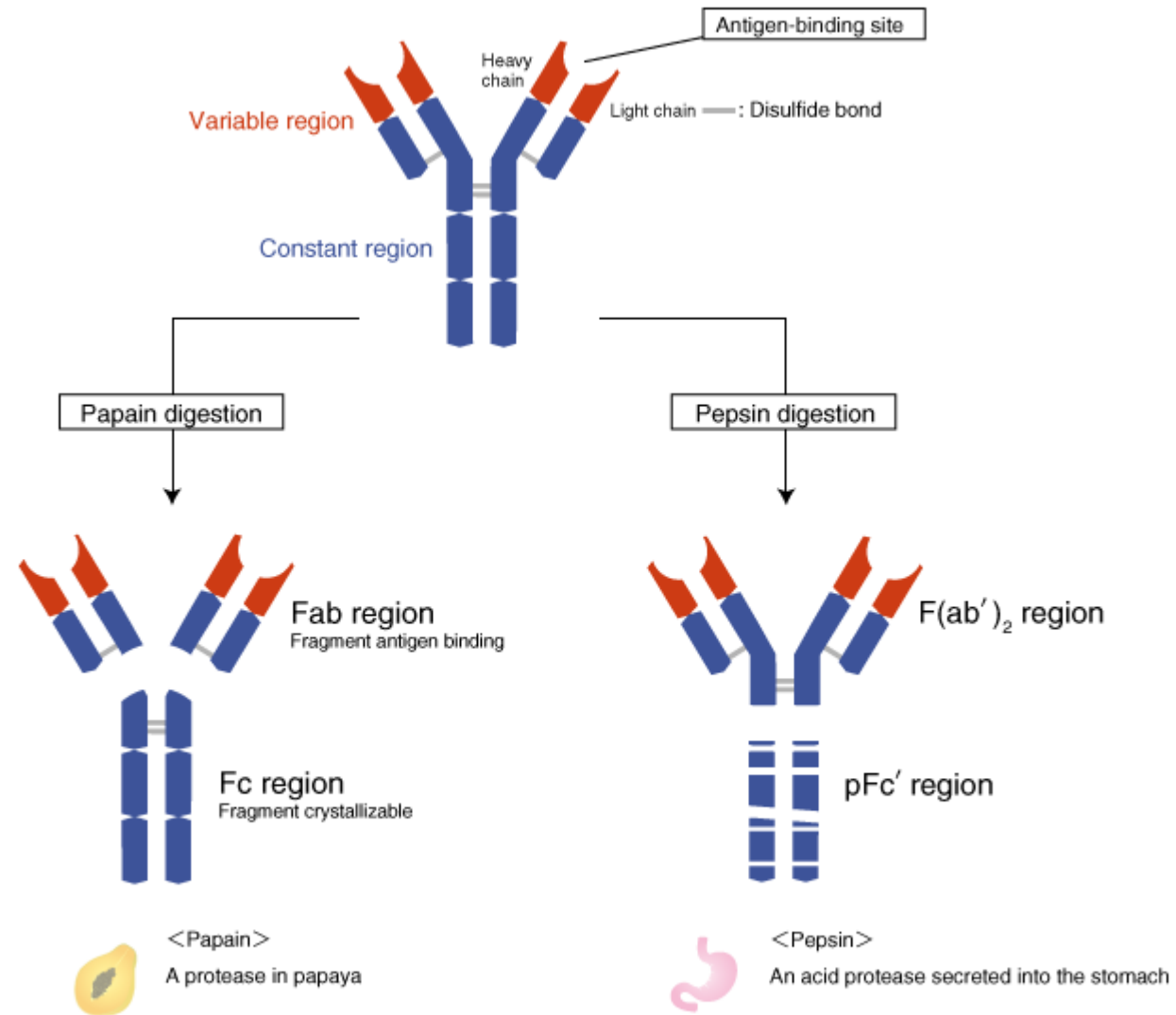
PROTEOLYSIS OF ANTIBODIES



Pepsin digestion results in a single F(ab')₂ fragment, with two antigen binding sites, and no surviving Fc fragment.

Papain digestion yields two Fab fragments (each having one antigen binding site) and one Fc fragment.

Figure 4.3 Janeway's Immunobiology, 8ed. (© Garland Science 2012)



Immunoglobulin Antigen Determinant

There are three major types of immunoglobulin antigen determinants:

Isotypes

Allotypes

Idiotypes

Immunoglobulin Antigen Determinant

1. Isotypes:

- The *isotype* of an immunoglobulin refers to the particular **constant region** of the light- or heavy-chain of the immunoglobulin.
- Immunoglobulins are classified on the basis of various heavy chain isotypes. Heavy chains are distinguished by the presence of heavy chain markers, such as μ , γ , α , δ and ϵ in the immunoglobulins IgM, IgG, IgA, IgD, and IgE, respectively.
- The light chains are also distinguished by isotype markers, such as κ and λ . Isotypes are present in all members of a species.

Characteristics of the Antibody Isotypes

	Light Chains	Heavy Chains	Other Chains
IgM	Kappa Chain Lambda Chain	Mu Chain	J Chain
IgD	Kappa Chain Lambda Chain	Delta Chain	
IgG	Kappa Chain Lambda Chain	Gamma ₁ Chain Gamma ₂ Chain Gamma ₃ Chain Gamma ₄ Chain	
IgE	Kappa Chain Lambda Chain	Epsilon Chain	
IgA	Kappa Chain Lambda Chain	Alpha ₁ Chain Alpha ₂ Chain	J Chain Secretory Component

Immunoglobulin Antigen Determinant

2. Allotypes

The *allotype* refers to allelic differences in both the variable and constant regions of immunoglobulin.

The allotype markers are present on the constant regions of light and heavy chains.

Allotypes are present in some but not all members of a species and are inherited in a simple Mendelian fashion.

Immunoglobulin Antigen Determinant

3. Idiotypes

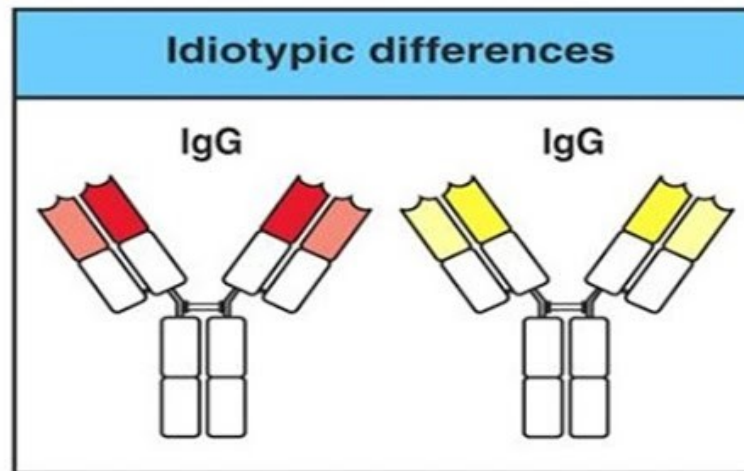
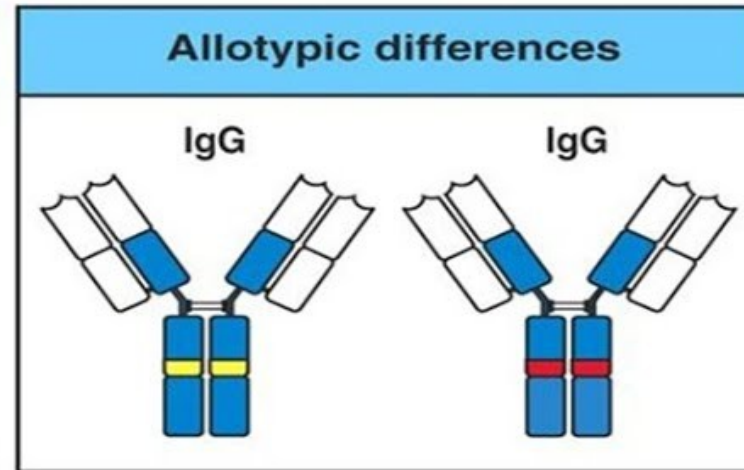
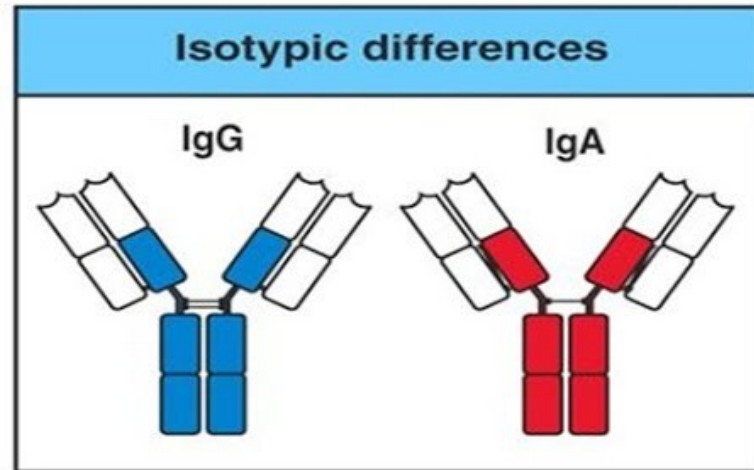
The *idiotype* refers to a specificity that is associated with the variable region.

Idiotypic markers are found on the hypervariable region of the immunoglobulin. Idiotypes are specific for each antibody molecule.

Isotypes, Allotypes and Idiotypes

Study Guide | Immunology

Study Guide | Immunology



Definitions:

Isotype:

- Different Fc
- Different type of Ab
- IgG, IgA, IgM, IgE, IgD

Allotype:

- Difference between species
- Most differences in Fc region.

Idiotypes

- Different specificities of the antibody molecules.
- Eg: each idioptye recognizes a different part of or different antigen

Activate Windows
Go to Settings to activate Windows.

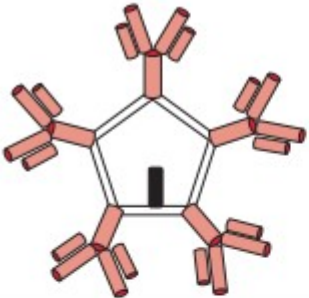
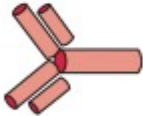
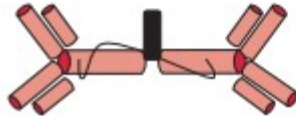
Biosynthesis of immunoglobulins

- B lymphocytes and plasma cells take part in the synthesis of immunoglobulins.
- Resting B cells synthesize only small amounts of immunoglobulins that mainly get incorporated into cell membranes.
- Plasma cells, the most differentiated B cells, are specialized to produce and secrete large amounts of immunoglobulins.
- The assembly of a complete Ig molecule is carried out either by associating one heavy and one light chain to form an H–L hemi-molecule, and then joining two H–L hemi-molecules to form a single complete molecule (H₂L₂), or by forming H₂ and L₂ dimers that later associate to form the complete molecule.

Metabolism of immunoglobulins

- Half-life ($T_{1/2}$) of immunoglobulin is one of the most commonly used parameters to assess the catabolic rate of immunoglobulins. The half-life corresponds to the time elapsed for a reduction to half of a circulating immunoglobulin concentration after equilibrium has been reached.
- The IgG is the immunoglobulin class with the longest half-life (average of 21 days), with the exception of IgG3. The IgG3 has a considerably shorter half-life (average of 7 days) that is nearer to that of IgA (5–6 days) and IgM (5 days).

Immunoglobulin classes

	IgM	IgM is pentameric. It is an effective first line defense against foreign bodies. IgM is produced in the primary immune response
	IgG	IgG is monomeric. It is an effective defense against extravascular compartments from foreign bodies and their components
	IgD	IgD is monomeric. It influences lymphocyte functions
	IgE	IgE is monomeric. It gives protection against intestinal parasites and is responsible for many of symptoms of allergy
	IgA	IgA is dimeric. It gives protection to mucosal surfaces

Immunoglobulin classes

1. Immunoglobulins G.

It has a half-life of 23 days—longest among all the immunoglobulins. IgG is the most abundant class of immunoglobulins in the serum, comprising about 80% of the total serum immunoglobulin.

The functions of IgG subclasses vary as follows:

1. IgG1, IgG3, and IgG4 are special because these are the only immunoglobulins with the ability to cross the placental barrier. They play an important role in protecting the developing fetus against infections.
2. IgG3, IgG1, and IgG2, in order of their efficiency, are effective in the activation of the complement.
3. IgG1 and IgG3 bind with high affinity to Fc receptors on phagocytic cells and thus mediate opsonization. IgG4 has an intermediate affinity for Fc receptors and IgG2 has an extremely low affinity.

Immunoglobulin classes

IgG shows the following biological activities:

- In response to infection, IgG antibodies appear late after the appearance of IgM antibodies, but persist for a longer period.
- It confers protection against the microorganisms that are present in the blood and tissues. It is distributed equally in the intra- and extravascular compartments.
- It is the only immunoglobulin that crosses the placenta; hence, it confers natural passive immunity to newborns.
- It takes part in complement fixation, and neutralization of toxins and viruses.
- It binds to microorganisms and facilitates the process of phagocytosis of microorganisms

TABLE 13-2**Comparison of various properties of immunoglobulins**

Characteristics	IgG	IgA	IgM	IgD	IgE
Structure	Monomer	Dimer	Pentamer	Monomer	Monomer
Percentage of total serum	80%	10–13%	5–8%	0.2%	0.002%
Location	Blood, lymph, and intestine	Blood, lymph, and B cell surface	Secretions	B cell surface, blood, and lymph	Bound to mast and basophil cell
Sedimentation coefficient	7	7	19	7	8
Molecular weight (kDa)	150	160	900	180	190
Carbohydrate (%)	3	8	12	13	12
Serum concentration (mg/mL)	12	2	1.2	0.03	0.00004
Half-life (days)	23	6–8	5	2–8	1–5
Heavy chain	$\gamma_1, \gamma_2, \gamma_3, \gamma_4$	α_1, α_2	μ	Δ	ϵ
Light chain	κ or δ	κ or δ	κ or δ	κ or δ	κ or δ
Complement binding	Classical pathway	Alternate pathway	Classical pathway	None	None
Placental transport	+	–	–	–	–
Present in milk	+	+	–	–	–
Seromucous secretion	–	+	–	–	–
Heat stability (56°C)	+	+	+	+	–
Binding to tissue	Heterologous	None	None	None	Homologous

Immunoglobulin classes

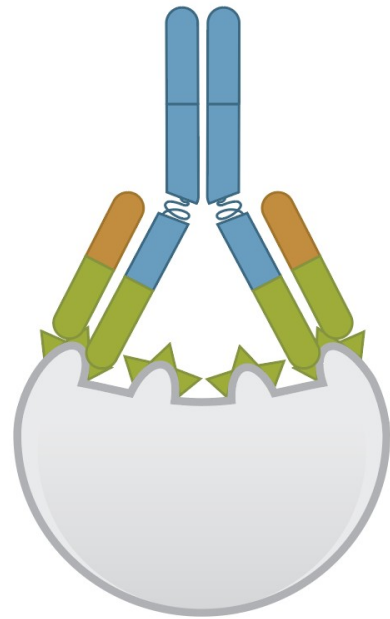
Immunoglobulin M

IgM constitutes about 5–8% of total serum immunoglobulins.

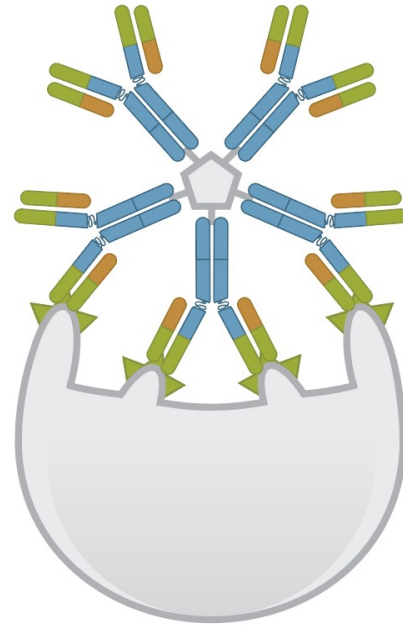
It has a half-life of 5 days.

IgM is basically a pentamer, composed of five immunoglobulin subunits (monomeric subunits, IgMs) and one molecule of J chain. Each monomeric IgM is composed of two light chains (κ or λ light chains) and two heavy chains (μ).

Two subclasses of IgM (IgM1 and IgM2) are described, which differ in their μ chains. IgM1 consists of μ 1 and IgM2 consists of μ 2 chains



IgG



IgM

Immunoglobulin classes

Monomeric IgM is expressed as membrane-bound antibody on B cells.

Being present on the membrane of B cells, IgM acts as the antigen-binding molecule in the antigen–antibody complex

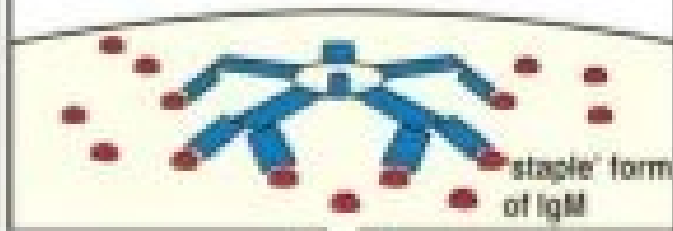
Immunoglobulin classes

- IgM shows the following biological activities:
 - Pentameric IgM is more efficient than other isotypes in binding antigens with many repeating epitopes, such as viral particles and red blood cells.
 - It is more efficient than IgG in activating complement. Complement activation requires two Fc regions in close proximity, and the pentameric structure of a single molecule of IgM fulfills this requirement.
 - IgM is the first immunoglobulin produced in a primary response to an antigen. The immunoglobulin confers protection against invasion of blood by microbial pathogens. Deficiency of IgM antibodies is associated with septicemia.

Pentameric IgM molecule binds to antigens on bacterial surface and adopts 'staple' form

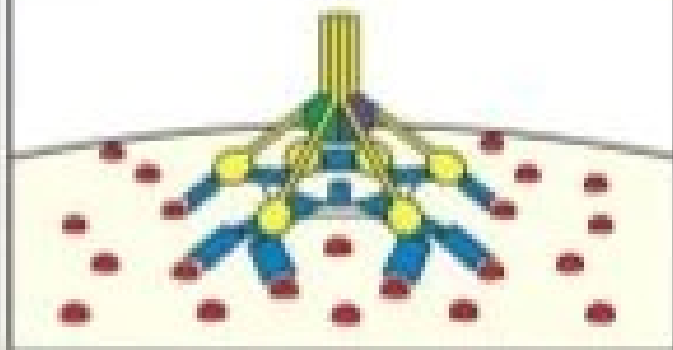


'planar' form of IgM

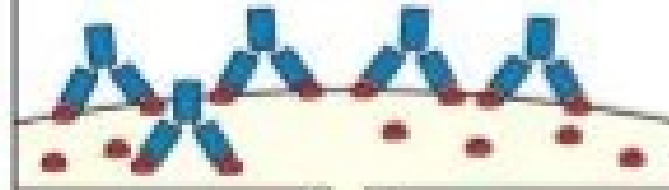


'staple' form of IgM

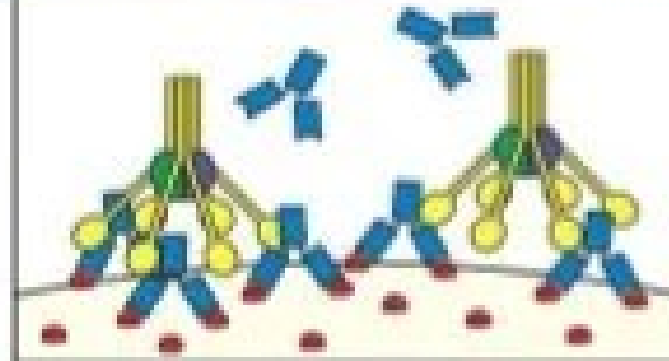
C1 binds to a single IgM molecule



IgG molecules bind to antigens on bacterial surface



C1q binds to two or more IgG molecules



Immunoglobulin classes

- IgM antibodies are short lived and disappear early as compared to IgG. The presence of IgM antibody in serum, therefore, indicates recent infection.
- It is also the first immunoglobulin to be synthesized by a neonate in about 20 weeks of age. IgM is not transported across the placenta; hence, the presence of IgM in the fetus or newborn indicates intrauterine infection. The detection of IgM antibodies in serum, therefore, is useful for the diagnosis of congenital infections, such as syphilis, rubella, toxoplasmosis, etc.

Immunoglobulin subclasses

Immunoglobulin A

IgA is the second major serum immunoglobulin, comprising nearly 10–15% of serum immunoglobulin. It has a half-life of 6–8 days.

IgA consist of heavy chain that confers class specificity on IgA molecules.

IgA occurs in two forms: serum IgA and secretory IgA.

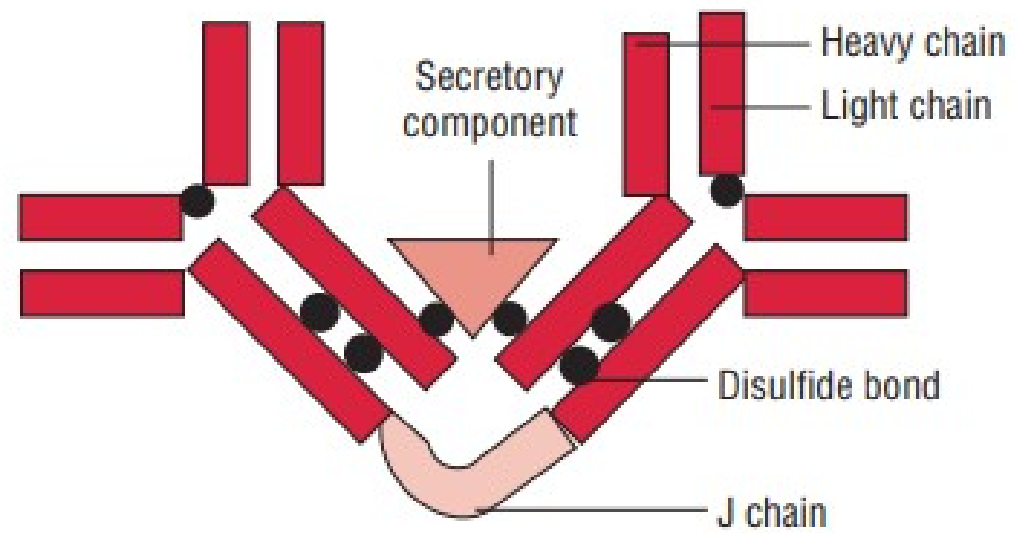


FIG. 13-3. Schematic diagram of immunoglobulin A (IgA).

Immunoglobulin classes

IgA shows the following biological activities:

- It protects the mucous membranes against microbial pathogens. It serves an important effector function at mucous membrane surfaces, which are the main entry sites for most pathogenic organisms. Because it is polymeric, secretory IgA can cross-link large antigens with multiple epitopes.
- Binding of IgA to bacterial and viral surface antigens prevents attachment of the pathogens to the mucosal cells, thus inhibiting viral infection and bacterial colonization. Complexes of IgA and antigen are easily entrapped in mucus and then eliminated by the ciliated epithelial cells of the respiratory tract or by peristalsis of the gut.

Immunoglobulin classes

- Breast milk contains secretory IgA and many other molecules that protect the newborns against infection during the first month of life. Because the immune system of infants is not fully functional, breast-feeding plays an important role in maintaining the health of newborns.
- Secretory IgA has shown to provide an important line of defense against bacteria (such as *Salmonella* spp., *Vibrio cholerae*, and *Neisseria gonorrhoeae*) and viruses (such as polio, influenza, and reovirus).

Classification of immunoglobulins

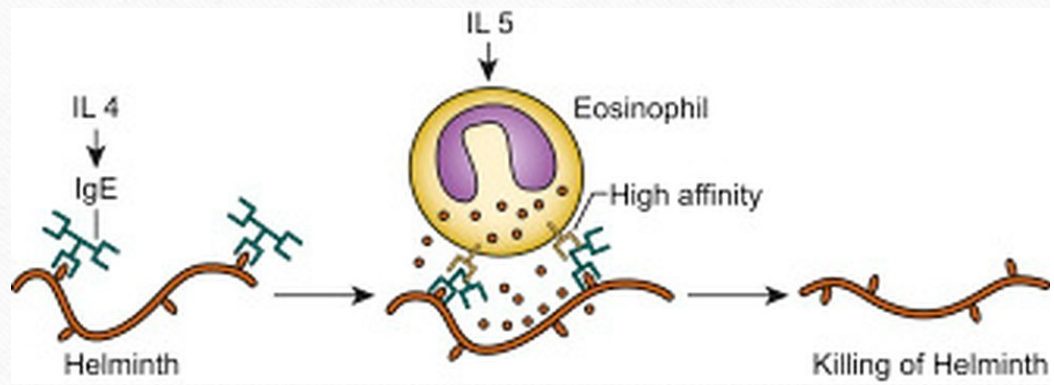
3. Immunoglobulin E

- IgE constitutes less than 1% of the total immunoglobulin pool. It is present in serum in a very low concentration (0.3 g/mL). It is mostly found extra vascularly in lining of the respiratory and intestinal tracts.
- IgE half-life of 2–3 days. Unlike other immunoglobulins that are heat stable, IgE is a heat-labile protein—easily inactivated at 56°C in 1 hour.
- IgE does not cross the placenta or fix the complement.

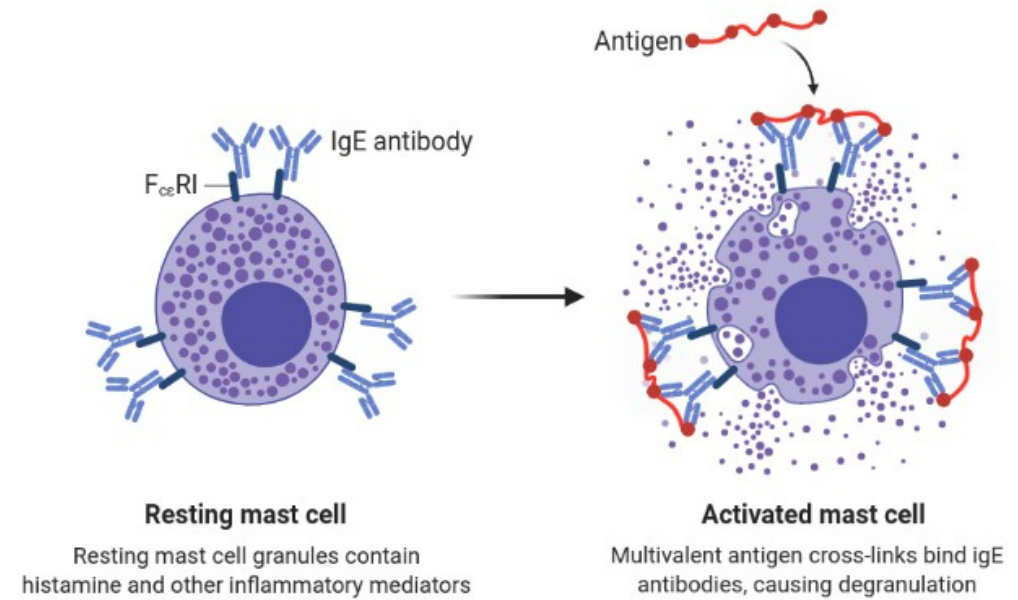
Immunoglobulin classes

IgE shows the following biological activities:

- IgE is also known as reaginic antibody that mediates the type I immediate hypersensitivity (atopy) reactions.
- IgE is responsible for the symptoms of hay fever, asthma, and anaphylactic shock. IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells. Cross-linkage of receptor bound IgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment—a process known as degranulation. As a result, varieties of active mediators are released and give rise to allergic manifestations.
- Localized mast-cell degranulation induced by IgE may also release mediators that facilitate a buildup of various cells necessary for antiparasitic defense.



IgE Cross-linking Induces Mast Cell Activation and Degranulation

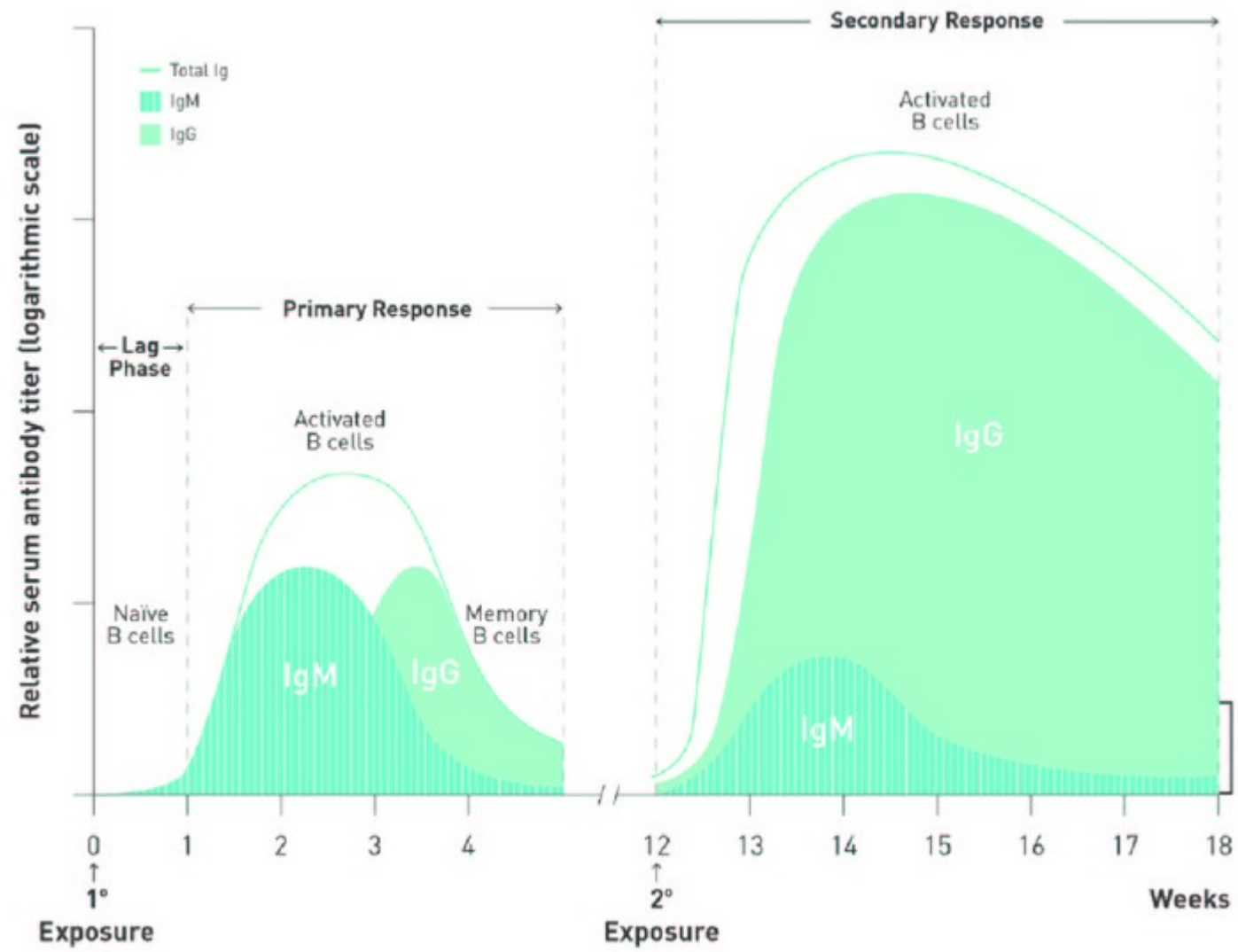


Immunoglobulin D

- IgD comprises less than 1% of serum immunoglobulins.
- The half-life of IgD is only 2–3 days.
- IgD has the basic monomeric structure
- IgD is very susceptible to the action of proteolytic enzymes at its hinge region.

TABLE 13-3**Role of immunoglobulins in human defense**

IgG	IgM	IgA	IgD	IgE
Enhances phagocytosis	Especially effective against microorganisms and agglutinating antigens	Localized protection on mucosal surfaces	Serum function not known	Allergic reaction
Neutralizes toxins and viruses	First antibody produced in response to initial infection		Present on B cells; and function in initiation of immune response	Possibly lysis of parasitic worms
Protects fetus and newborn				





The biology of B-lymphocytes

Malak Alshaikhali

MD, Al-Quds University

MSc Integrated Immunology, University
Of Oxford.

Learning outcomes

- Understand B-cells development.
 - Brief comments on tolerance.
- Understand TD antibody responses.
- Understand TI antibody responses.
- Understand membrane bound proteins and intracellular signaling pathways associated with B cell.

Definitions

- **Primary lymphoid organs:** bone marrow and thymus, where lymphocytes are produced.
- **Secondary lymphoid organs:** spleen, lymph nodes and mucosal lymphoid tissues.
- **Lymphopoiesis:** the production of new lymphocytes which take place in central lymphoid organs, that is BM for B cells and thymus gland for T cells.

Definitions

- **Genes**

The basic unit of heredity and a sequence of nucleotides in DNA that encodes the synthesis of a gene product, either RNA or protein

- **Allele**

Is one of two, or more, forms of a given gene variant.

Definitions

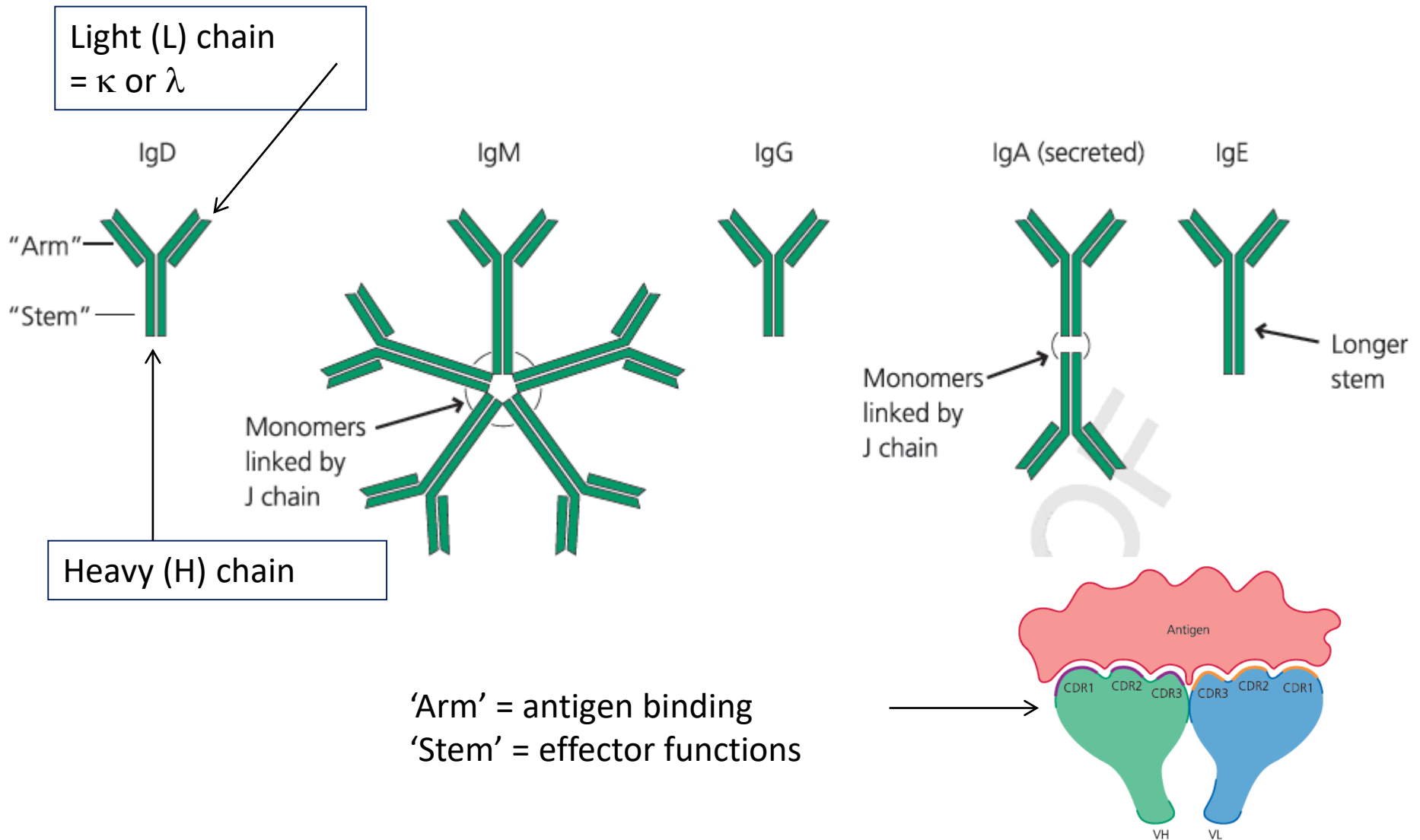
- **Cytokines** -*Cyto*: cell and *Kines*: movement

Small secreted proteins mediate and regulate immunity, inflammation, and haematopoiesis produced *de novo* in response to an immune stimulus

- **Chemokines**

Small cytokines produced by many types of cells that influence the movement of leukocytes including B and T lymphocytes, many are proinflammatory.

Antibodies



Stages of B-cells development.

- **Maturation**-Stem cells to mature, naïve B cells.
- **Activation**-Ag binding; initiation of cell changes.
- **Differentiation**-Cell division and changes into effector B cells (plasma cells) and memory B cells.

B-cells production and maturation

- B cells production starts in fetal life : fetal liver, spleen and yolk sac, then continues in the bone marrow.
- New B-cells are continuously produced throughout life; does not wane as does T cell production.
- The stages in B-cell development are defined mainly by the successive steps in the assembly and expression of functional antigen receptor genes.
- **Checkpoints:** the process of gene rearrangement is monitored to reinforce the requirement that each B cell expresses just one receptor specificity.

B-cells origins

- Multipotent hematopoietic stem cells (HSCs) in the bone marrow give rise to common lymphoid progenitor stem cells (CLP)
- CLP cells give rise to all lymphoid cells, T-,B- and NK-cells.
- In lymphocyte development, cells become committed first to the lymphoid lineage, and then to either the B -cell or the T-cell lineages (depending on signals received) .

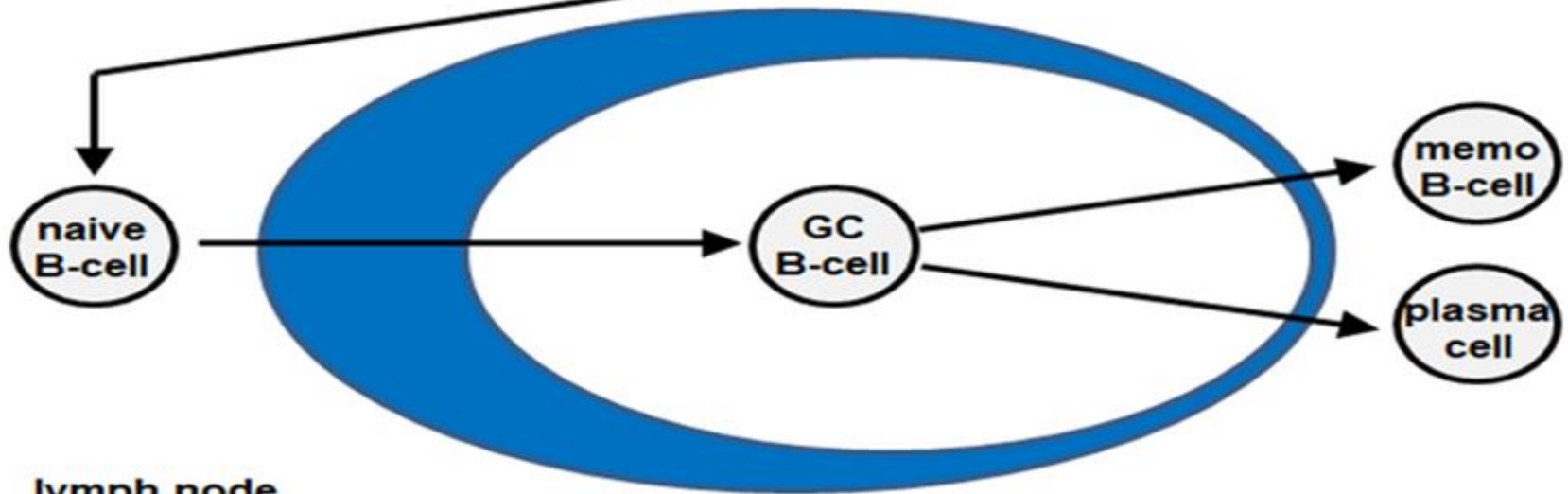
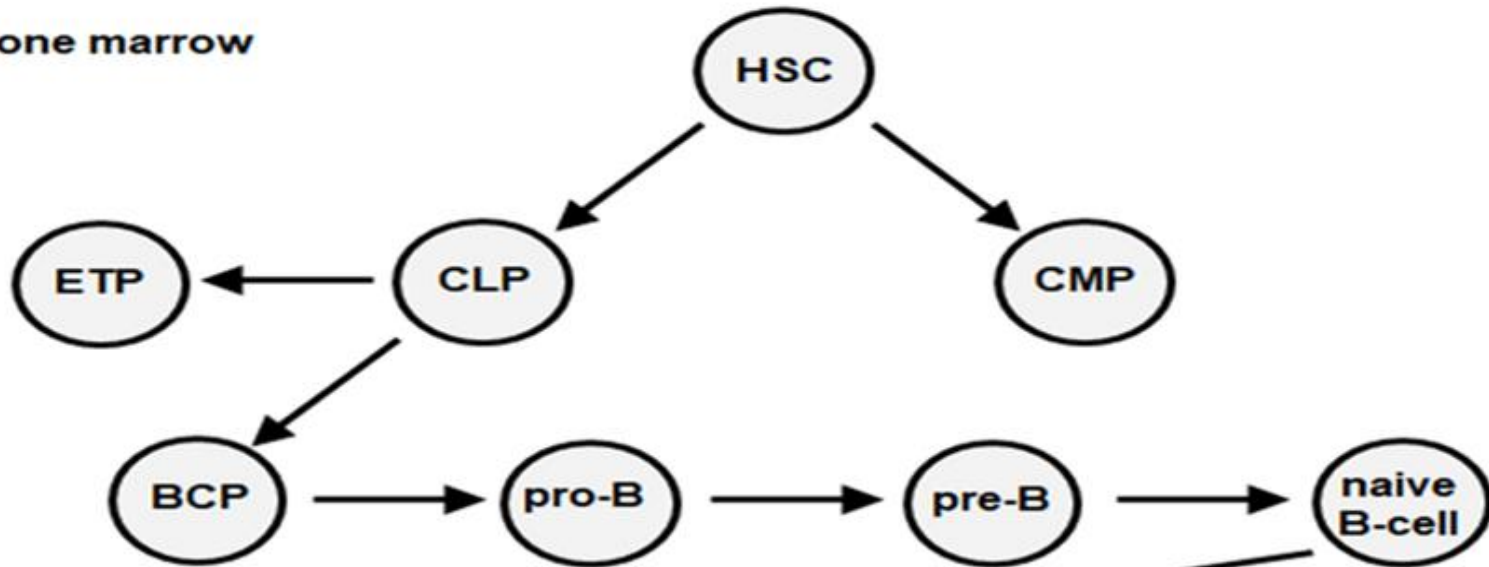
B-cells origins

- Developing lymphocytes receive differentiation signals from specialized non-lymphoid connective-tissue in the BM called **stromal cells**.
- These cells are in intimate contact with the developing lymphocytes.

B-cells origins

- The contribution of the stromal cells is twofold:
 - **First:** they form adhesive contacts with the developing lymphocytes.
 - **Second:** they provide cytokines and chemokines that control lymphocyte differentiation and proliferation and survival e.g. BAFF and IL-7.

bone marrow



Hematopoietic stem cell (HSC), common myeloid progenitors (CMP), common lymphoid progenitors (CLP), early T-cell progenitors (ETP), Bcell progenitor (BCP), germinal center (GC)

Pro-B cells

- The CLP gives rise to the earliest committed B-lineage cell, the *pro-B cell*.
- B-cell development begins by rearrangement of the heavy-chain locus.
- *V(D)J heavy chain genes* rearrangement is mediated by the V(D)J recombinase.
- The early steps of immunoglobulin genes is rearranging D_H segment to J_H segment.

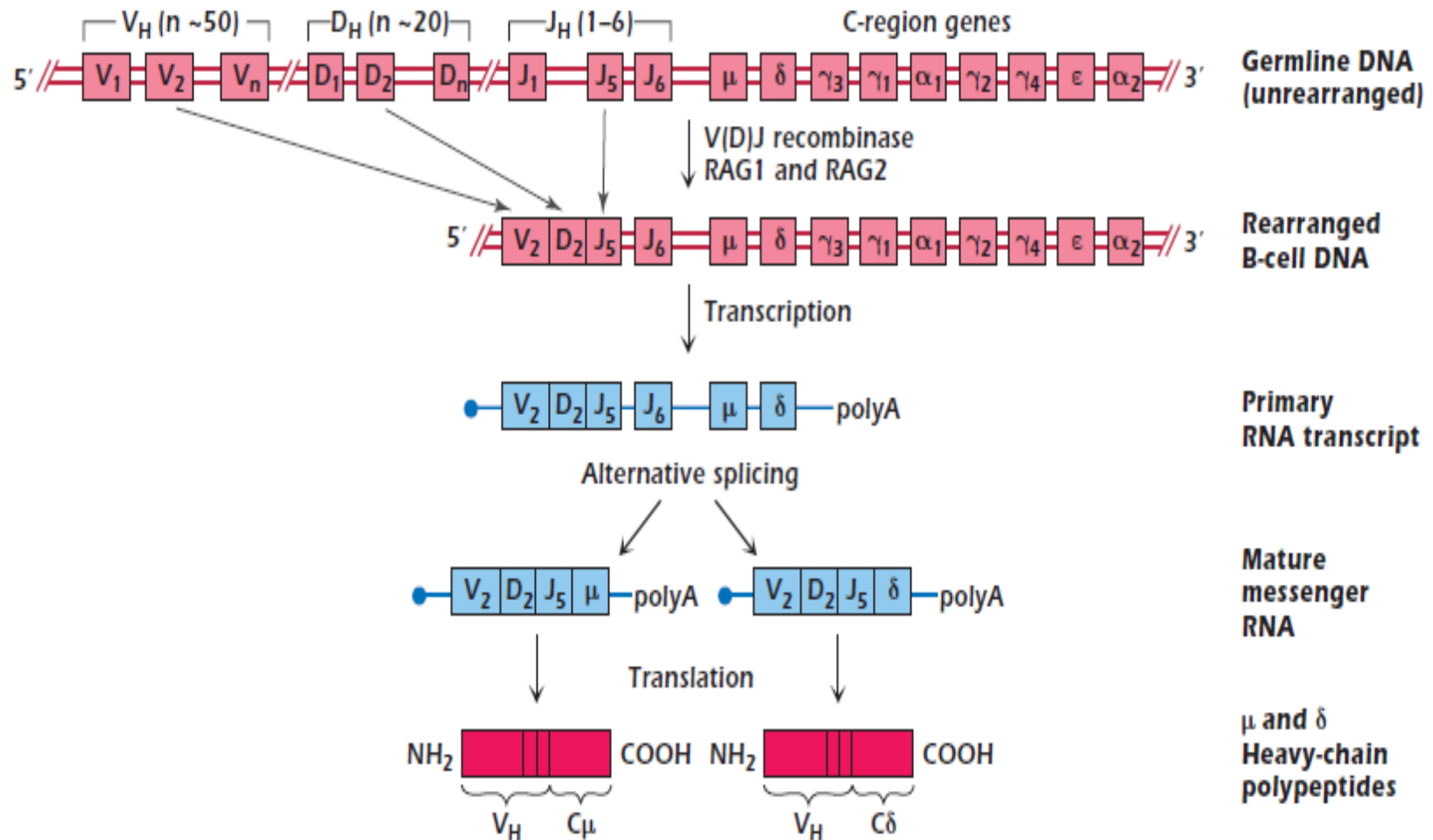
Pro-B cells: markers expression

- Pro-B cells starts to express CD19 and CD10.
- CD19 is an exclusive B cell marker expressed throughout all B-cell stages, except plasma cells.

Pre-B cell

- At this stage, the Ig *heavy chain* gene locus undergoes a second rearrangement.
- V_H gene segment joins the D_HJ_H segment, forming VDJ unit.
- If the rearranged VDJ genes is productive, pre-B cell synthesizes a μ chain.
- Gene rearrangement occurs at one chromosome at a time until functional μ chain is produced.

Heavy chain recombination



Allelic exclusion

- **Definition:**

The rearrangement of Ig chains genes occurs in sequential manner, that is genes from only one chromosome are used to make a heavy chain and genes from one chromosome to make a light chain at a time.

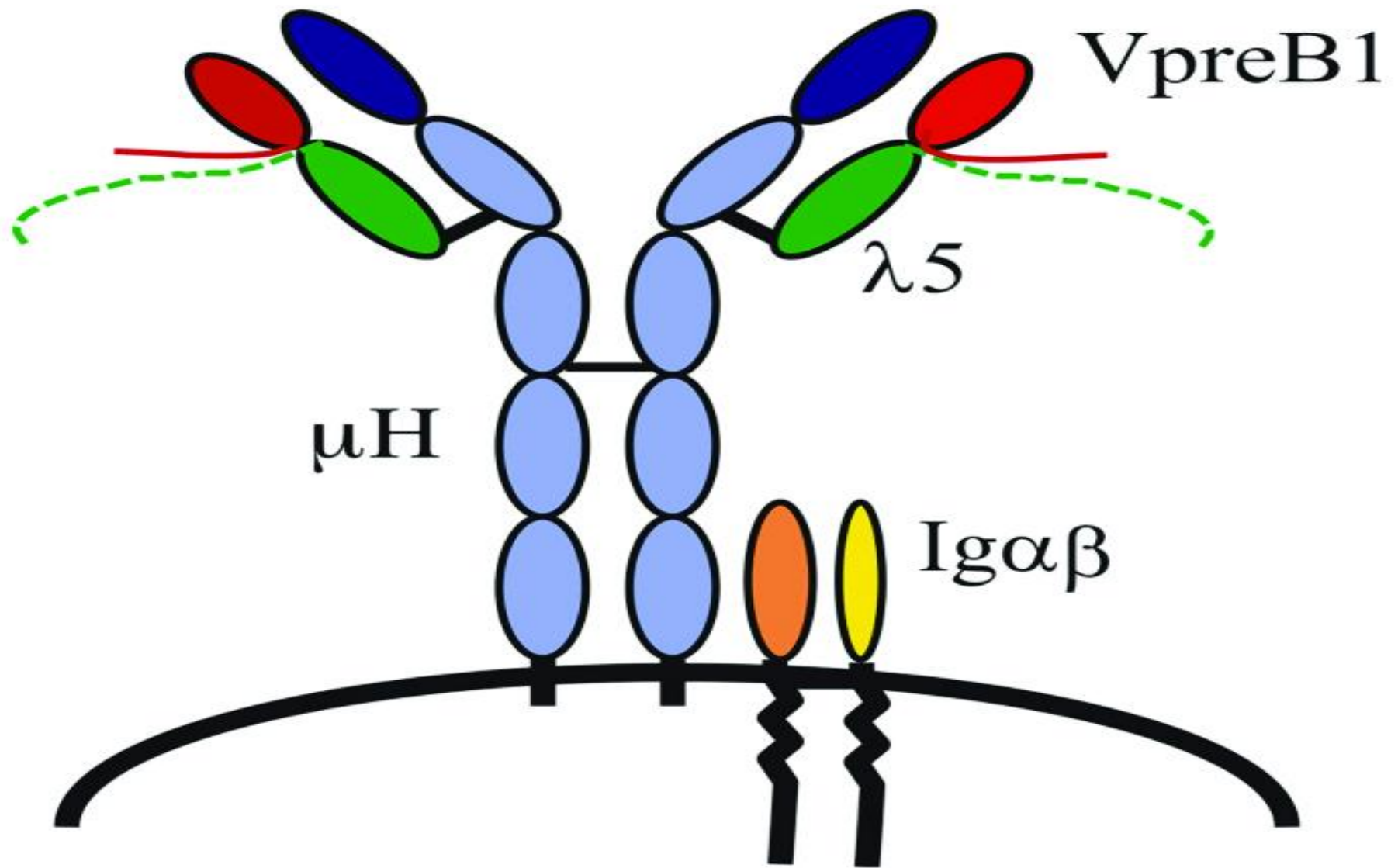
- This process ensures that an individual B cell expresses an Ig molecule with only one single antigenic specificity.

Pre-B cells: pre-B cell receptor

- Pre-B cells express Pre-B cell receptor.
- The expression of the pre-BCR is a key early developmental **checkpoint** in the B cell lineage.
- If the cell does not make a pre-BCR because no heavy-chain rearrangement is productive the cell dies by *apoptosis*.

Pre-B cells: pre-B cell receptor

- The pre-BCR consists of the μ chain and two sets of tightly associated proteins:
 1. The surrogate light chains VpreB and $\lambda 5$.
 - Only present at this stage of B-cell development.
 2. Ig α and Ig β heterodimer
 - Present from pre-B cell stage to memory cells.
 - Perform signals transduction function through *ITAMs*.



The pre-B cell receptor (pre-BCR). A pre-BCR is assembled from antibody heavy (μ H) and surrogate light chains together with the signaling molecules Ig α and Ig β . The SL chain is composed of VpreB and λ 5

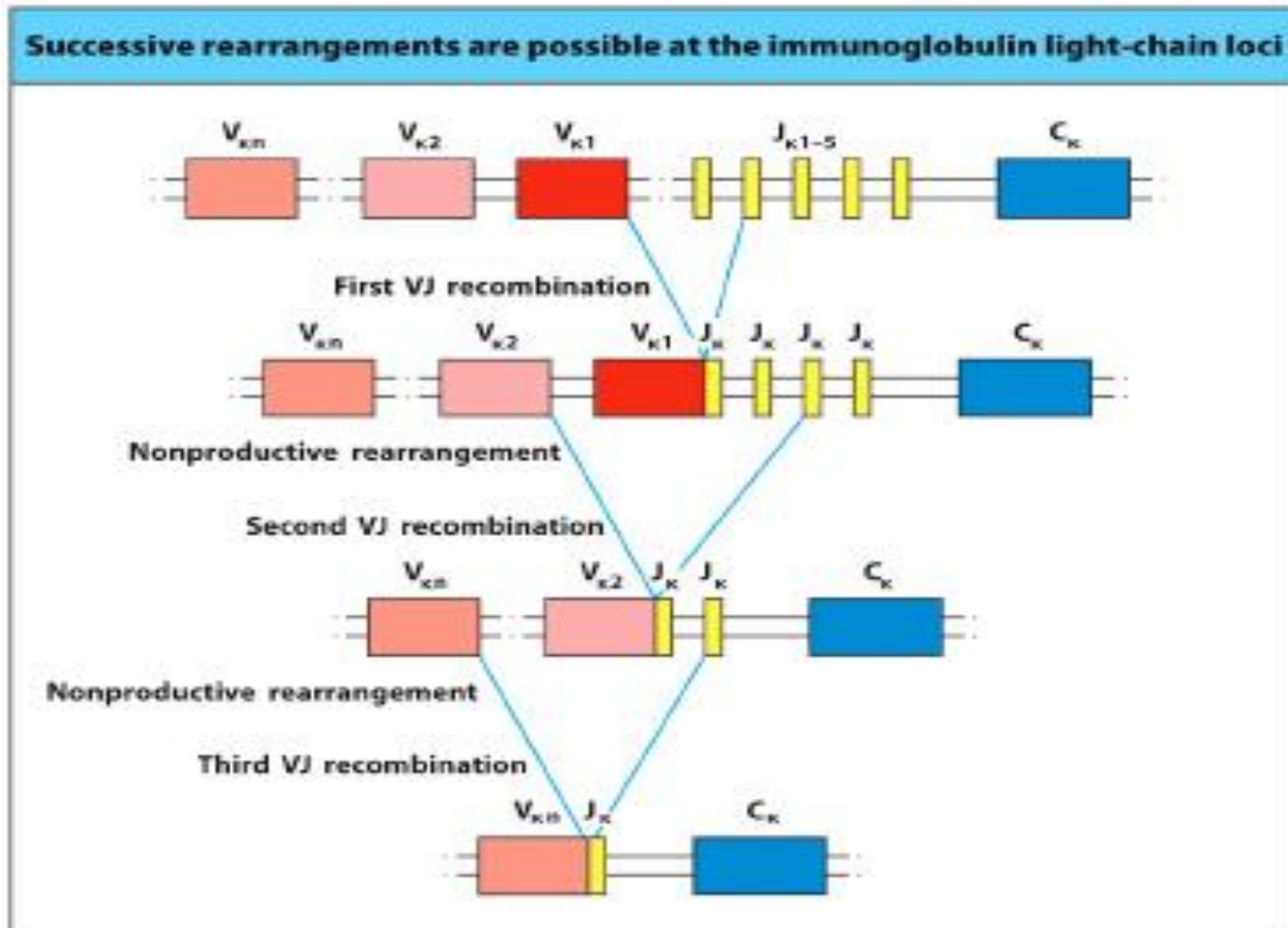
Pre-B cells: pre-B cell receptor

- ***Bruton's tyrosine kinase*** (Btk) transmit signals from pre-BCR to the nucleus essential for transition of pre-B cells to the next stage in B-cell differentiation
- Mutation in *BTK* gene cause the immunodeficiency condition, ***X-linked agammaglobulinemia***, in which B-cell differentiation is arrested at the pre-B-cell stage.

Pre-B cells: light chain synthesis

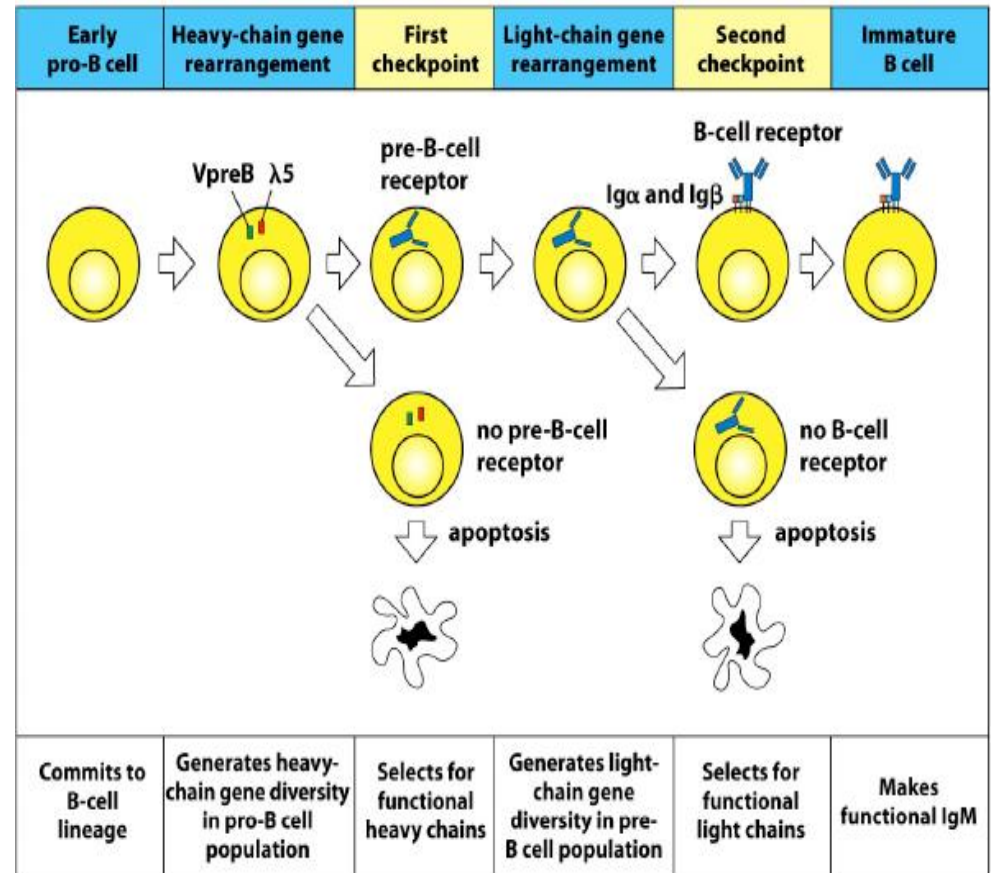
- Signals transduced through $Ig\alpha/Ig\beta$ conducts the following messages:
 - Heavy chain Ig genes were successfully rearranged into functional μ chain.
 - Surrogate light chain synthesis is shut off and light chain gene are rearranged to form functional light chains.
 - The same principle of allelic exclusion applies*.

Pre-B cells: light chain synthesis



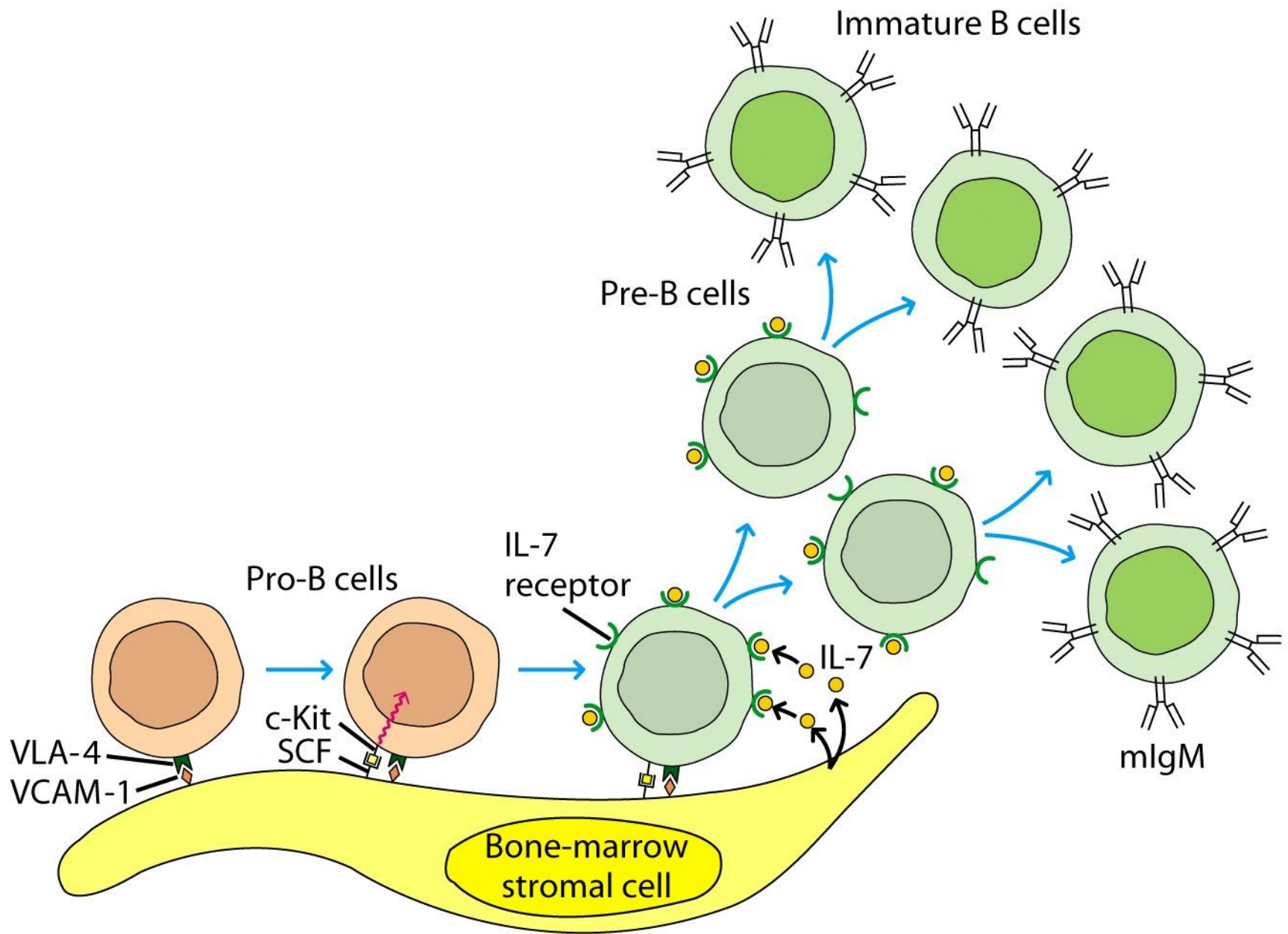
Checkpoints during Ig chain synthesis

- Check 1: pre-B cell receptor with correct VDJ fused to μ heavy chain and associated with surrogate light chain.
- Check 2: BCR with correctly rearranged light chain and μ heavy chain on cell membrane.



Immature B cells

- Light chains pair with μ chains to form IgM.
- IgM is expressed on B- cell surface to form BCR.
- Immature B-cells express CD20.
- Immature BCR can bind antigens (both foreign and self).
- Central tolerance process and negative selection of auto-reactive B-cells occur at this stage.
- BM stromal cells: present self-Ags for B-cells selection.



B cells – problems & solutions

- Problem
 - BCR are generated essentially ‘at random’ – many could potentially recognise ‘self’ antigens (i.e. autoreactivity, perhaps leading to autoimmunity)
- Solution
 - ‘Negative selection’
 - Immature B cells that react with high avidity to ‘self’ antigens die (clonal deletion), are rendered impotent (anergy) or ‘given another chance’ (receptor editing): all part of central tolerance

Central tolerance

- **Negative selection:** Lymphocytes with strongly self-reactive receptors must be eliminated to prevent autoimmune reactions.
- This process is one of the ways in which the immune system is made self-tolerant.
- **Positive selection:** Developing lymphocytes whose receptors interact weakly with self antigens, receive a signal that enables them to survive.

Central tolerance

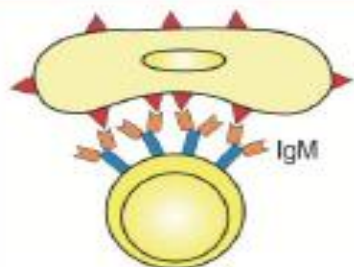
- **Receptor editing:**

Instead of deletion, immature B-cells reactive to self-Ag undergo a second VDJ gene rearrangement at the unrearranged light-chain gene segments (V-J).

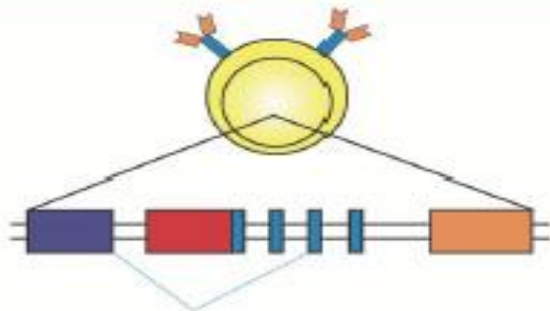
☐ Receptor editing does not occur on the heavy chain.

- **Anergy:** inducing unresponsiveness of self-reactive B-cells.

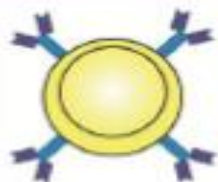
Strong ligation of IgM by self antigen



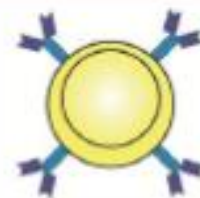
Arrest of B-cell development and continued light-chain rearrangement: low cell-surface IgM



A new receptor specificity is now expressed



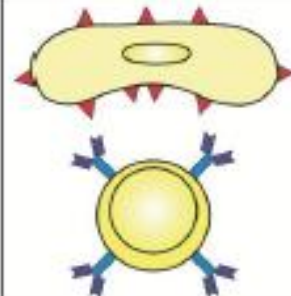
A new receptor specificity is now expressed



If the new receptor is still self-reactive, the B cell undergoes apoptosis



If the new receptor is no longer self-reactive, the immature B cell migrates to the periphery and matures



Transitional B cells

- Cells that survived negative selection and ready to leave the BM.
- They start to express IgD together with IgM at the cell surface.
- They express a receptor for the cytokine BAFF.
- BAFF is a survival signal for transitional B cells, and cells in later stages in the B cell lineage.

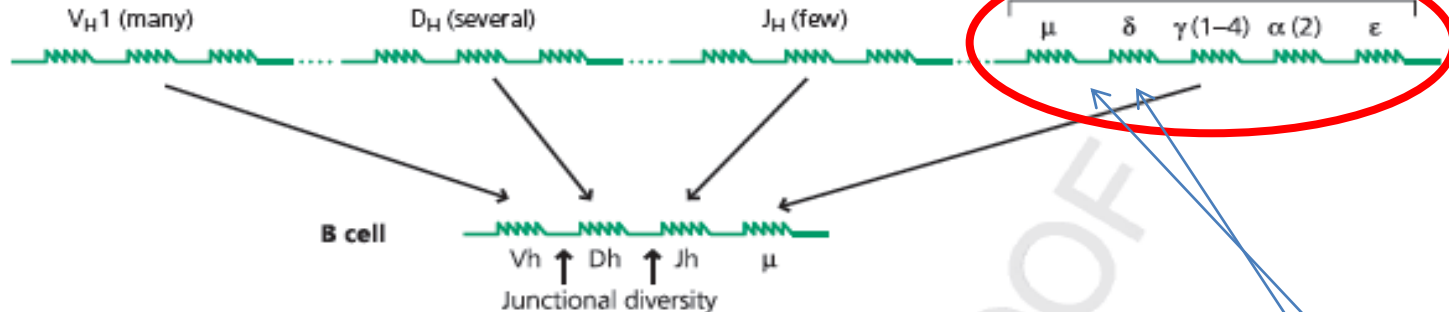
Mature B Cells

- IgM+ IgD+ mature B cell (resting B-Cells).
- Naïve mature B-cells: cells that did not encounter an antigen.
- **Follicular mature B-cells:** they recirculate through the blood to the secondary lymphoid organs where they survey for specific Ag.
- **Marginal zone B-cells:** mature non-recirculating B-cells that become resident in the spleen marginal zone.

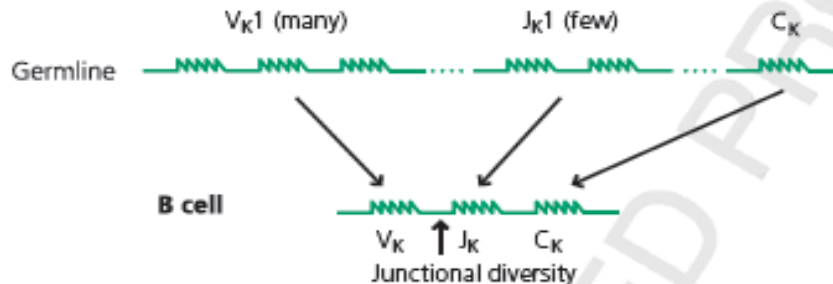
Somatic recombination – Ig genes

Heavy chain gene segments

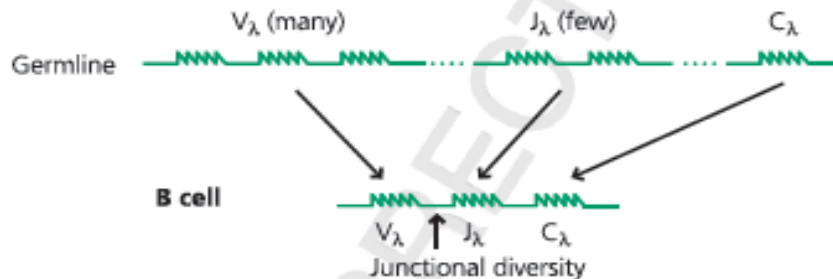
Germline

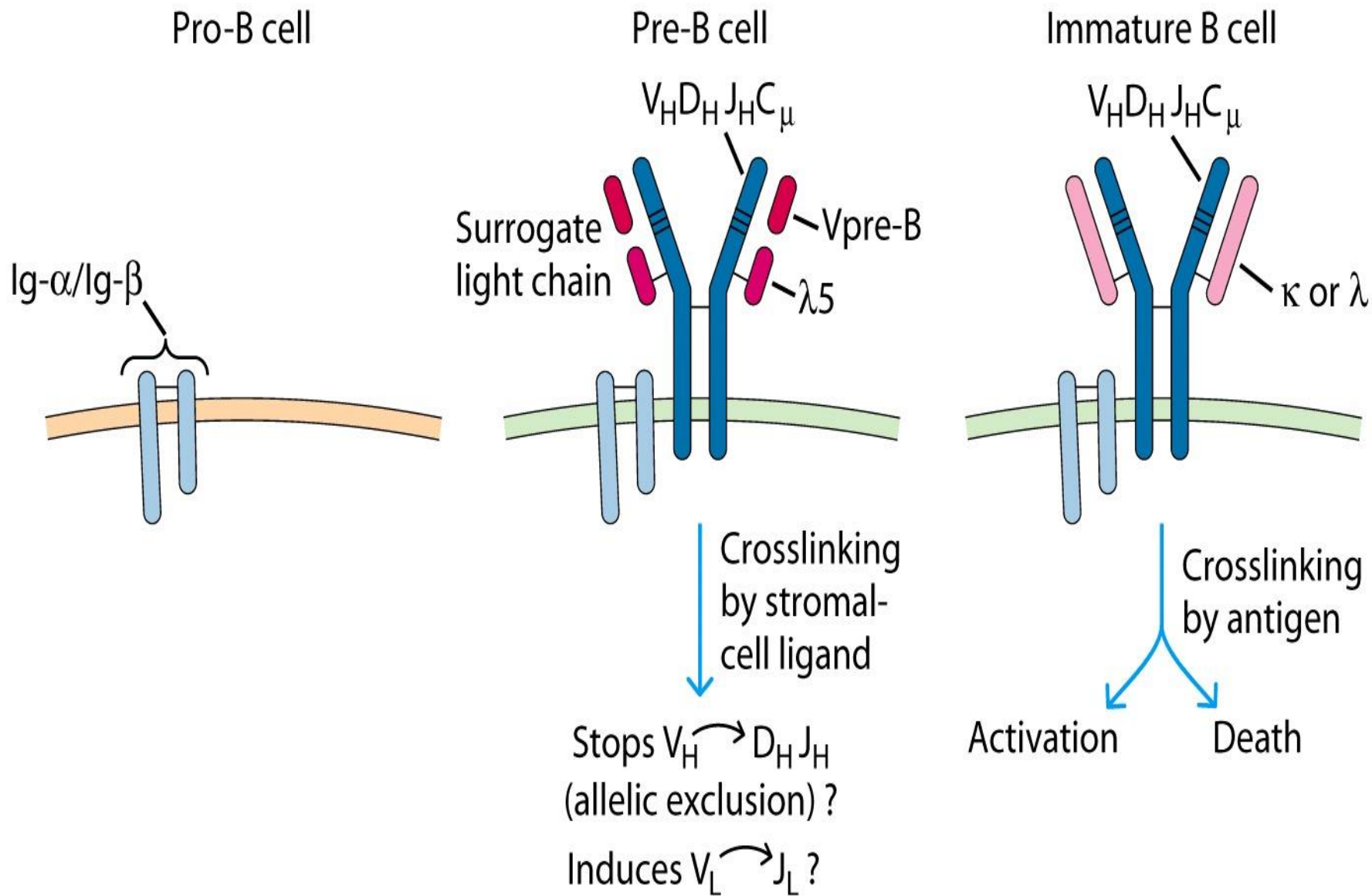


Light chain (kappa) gene segments



Light chain (lambda) gene segments



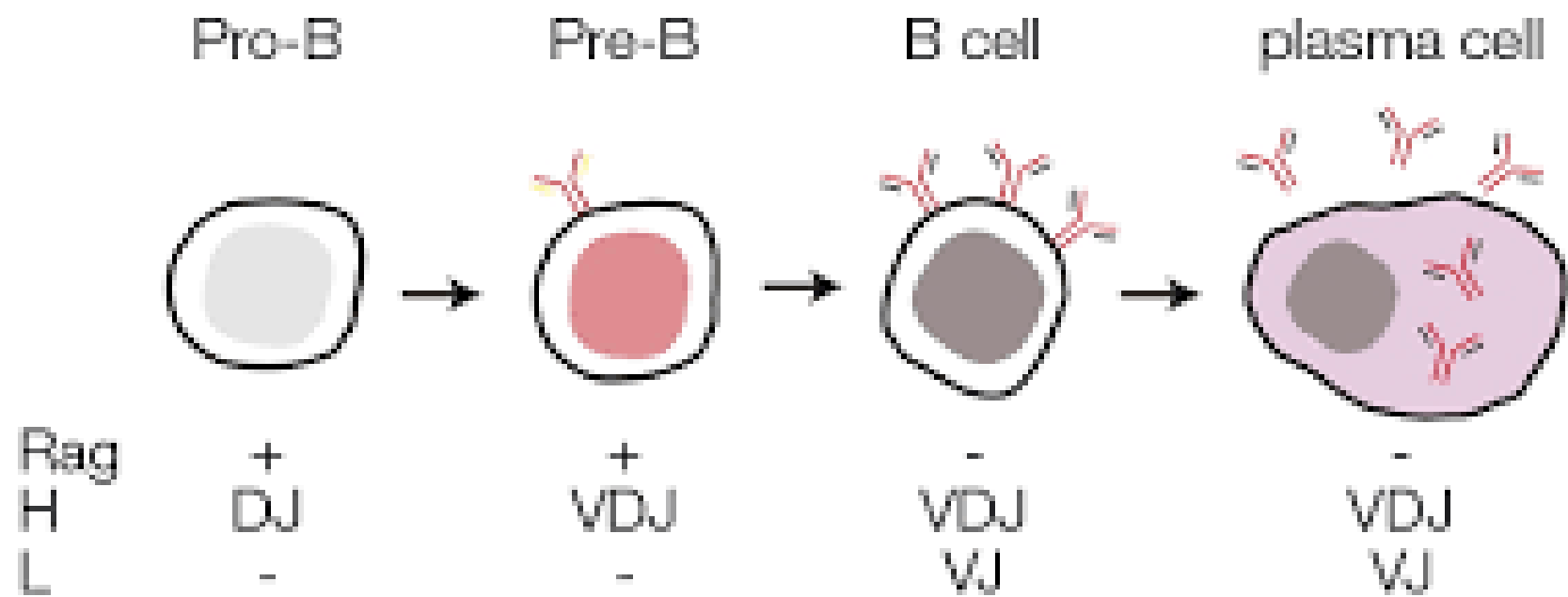


Plasma Cells

- The terminally differentiated stage of B-cell that synthesize and secrete Igs.
- They secrete Abs of single Ag and isotope specificity*.
- They do not express a membrane form of Ig.
- They express-along with memory cells-CD27 and lose expression of CD10, CD19 and CD20.

Plasma cells

- Short-lived and long-lived.
- Produced at LN, spleen and MALT.
- Respond to antigens in TD and TI manner.
- MALT also contain plasma cells.
- They secrete dimeric IgA into secretions of the gut, respiratory tract, tears and saliva.



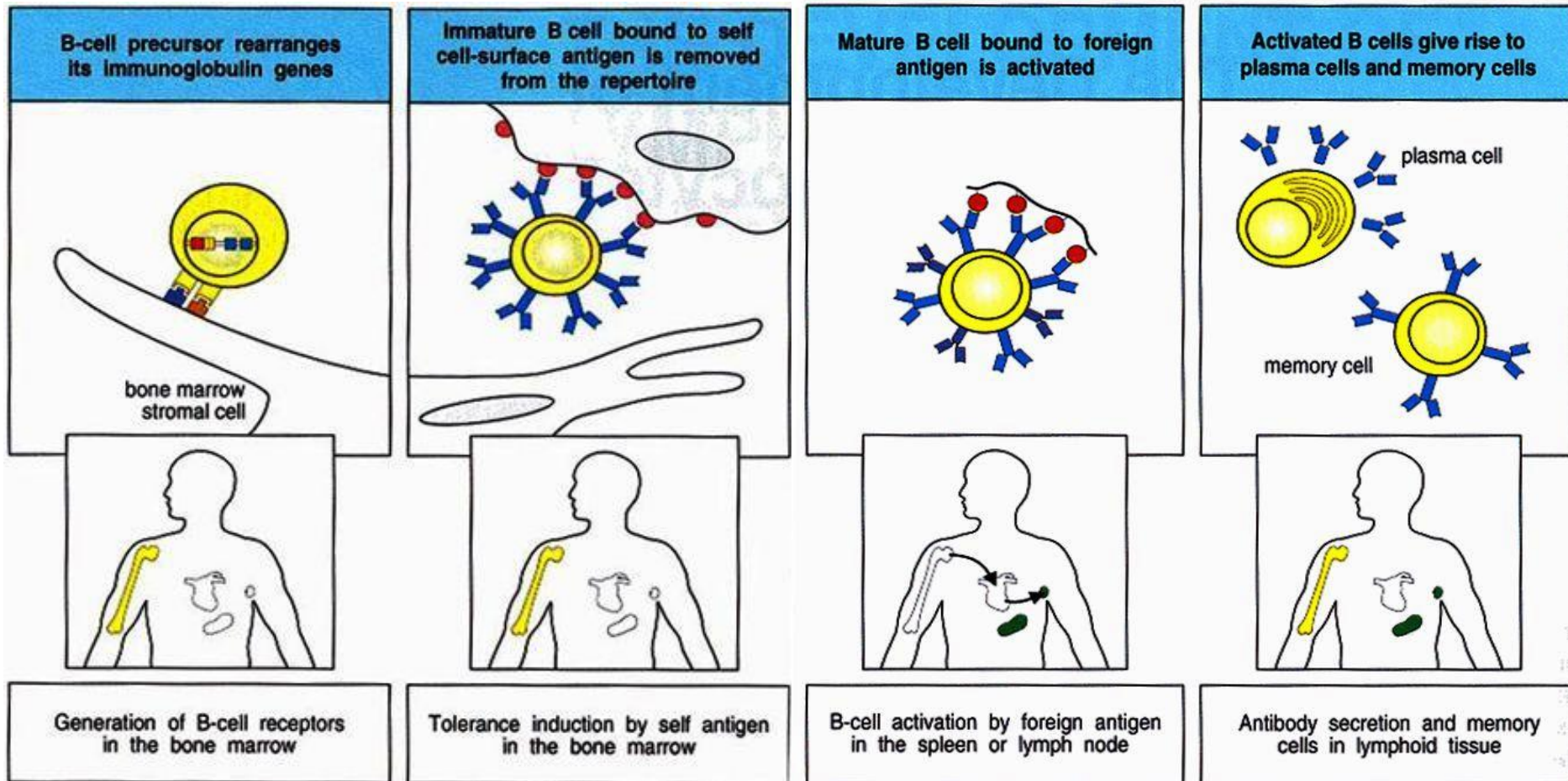
Long-lived Plasma cells

- Develop in the GS of LNs and spleen.
- Migrate back to BM and become resident and fighting infections.
- Mainly secrete IgG, monomeric IgA and some secrete IgE.

Memory B Cells

- Develop in germinal centers of secondary organs after TD-antigen activation of the mature B cell.
- Express IgM along with other isotypes- IgG, IgA, IgE, but don't express IgD.
- They don't circulate as naïve B cells, they reside in tissues.
- Non-proliferating and when activated becomes plasma cells.

Life cycle of B cells

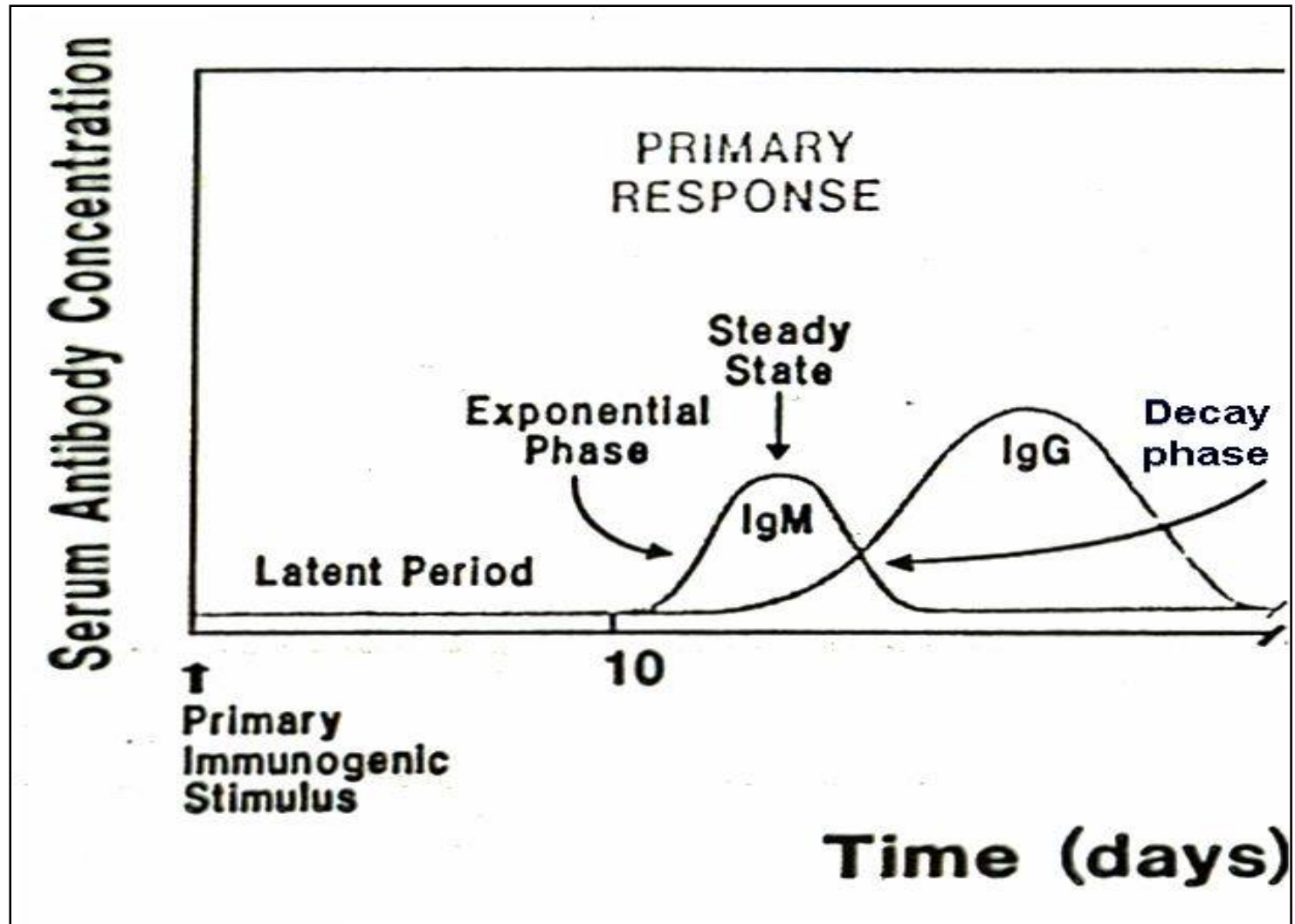


* 'Conventional' B cells

TD antibody responses

- Thymus-dependent (TD) because they require helper T cells.
- Polyclonal in nature:
 - Secrete antibodies of different isotypes and of specificities to different epitopes of the same antigen.
- Production site: germinal center of the spleen, LNs and in the MALT.
- T-cells help is generally needed for antibody responses against *protein-containing* antigens.

T-dependent responses



Antibody synthesis

- The main function of B-cells/plasma cells is to produce antigens.
- TD responses: antibody response to antigens require T-cells help — CD4+ helper T-Cells.
- ***Cognate T and B cells***: The CD4+ T and B cell that cooperate in the response to TD antigens are both specific for the same antigen (linked recognition).
- They find each other at sites of SLO e.g. LN

Antibody synthesis

- Once cognate B-and T-cells are activated by the specific antigen, they interact with each other at the boundary of the follicle and T-cell area of the node.
- Here, B-cells act as APC for T-cells.
- The interaction between CD4+ T cell and the B cell lasts several hours~ 48hrs.
- The result: B cell differentiate into plasma cell that synthesizes immunoglobulins.

TI antibody responses

- ***Thymus-independent*** (TI) antigens: a group of antigens that stimulate B cell responses without requiring CD4+ Th cells.
- Antigens do not require T-cell help for the B cell to make antibody.
- Antibody response to non-peptide antigens e.g. polysaccharide of bacterial capsules.
- Provide early protection against bacterial infection- agglutination and complement activation.

B-cell membrane proteins

☐ Stage specific surface marker expression.

- **CD10** is expressed by pro-B and pre-B cell and GC B cells.
- **CD19** is expressed on all cells in the B-cell lineage from the pro-B to memory B cell, but not the plasma cell.
- **CD20** is expressed on all cells from the immature B cell to the memory B cell but not the plasma cell.
- **CD27** is expressed exclusively on memory and plasma cells.
- **CD5** characterizes the B-1 subset of B cells.

☐ **Membrane Ig** all B cells except pro-B-cells and plasma cells

☐ **MHC class II** molecules are expressed on all cells in the B-cell lineage except pro-B cells.

☐ **Cytokines receptors:** eg IL-4 and INF- γ

Membrane Ig-Signal transduction

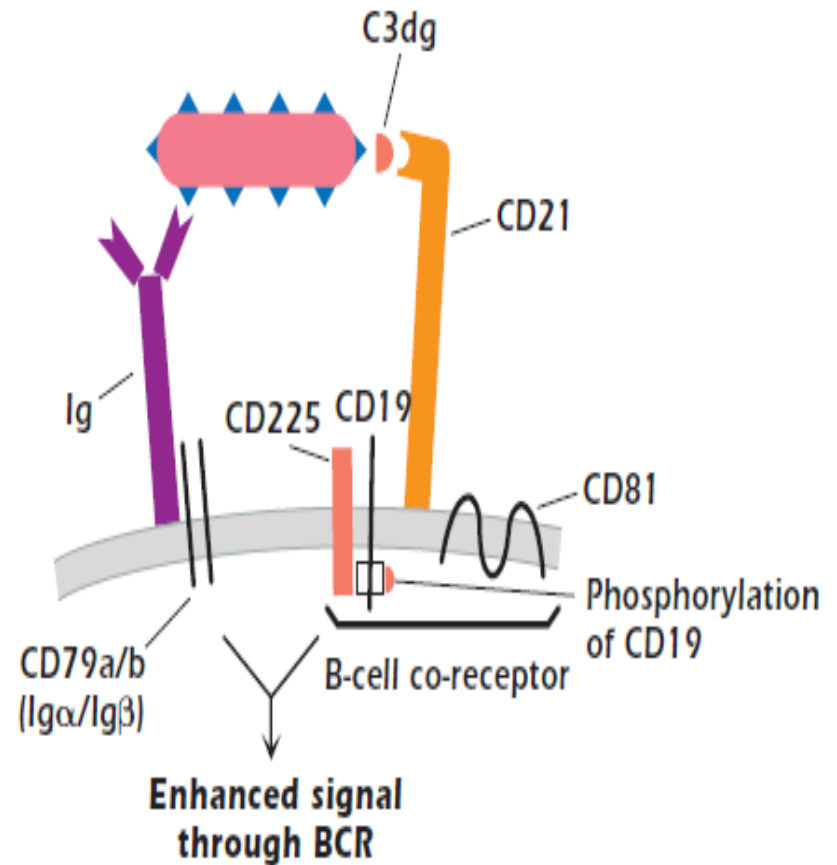
- Membrane-associated immunoglobulin binds antigen.
- Membrane Ig are associated with signal transduction molecules: $Ig\alpha/Ig\beta$ and B-Cell Co-Receptor.
- **$Ig\alpha/Ig\beta$ dimer(CD79a, CD79b):**
 - Tightly associated in the membrane of B cells with Ig heavy chains to form the BCR.
 - Transmit activation signals inside the cell.

B-Cell Co-Receptor

- Lies close to the BCR complex (Ig molecule+ Ig α /Ig β)
 - Consists of four molecules—CD19, CD21, CD81 (also known as TAPA-1), and CD225 (or Leu13).
 - Analogous Co-receptors on T-cells: CD4&CD8.
 - **Function:** enhances the signal transmitted through the BCR after antigen stimulation which reduces the threshold for B cells activation.
- ☐ Less antigen is needed to stimulate an antibody response if the co-receptor is activated together with the BCR (~100–1000x less) compared to activation through the BCR alone.

B-Cell Co-Receptor

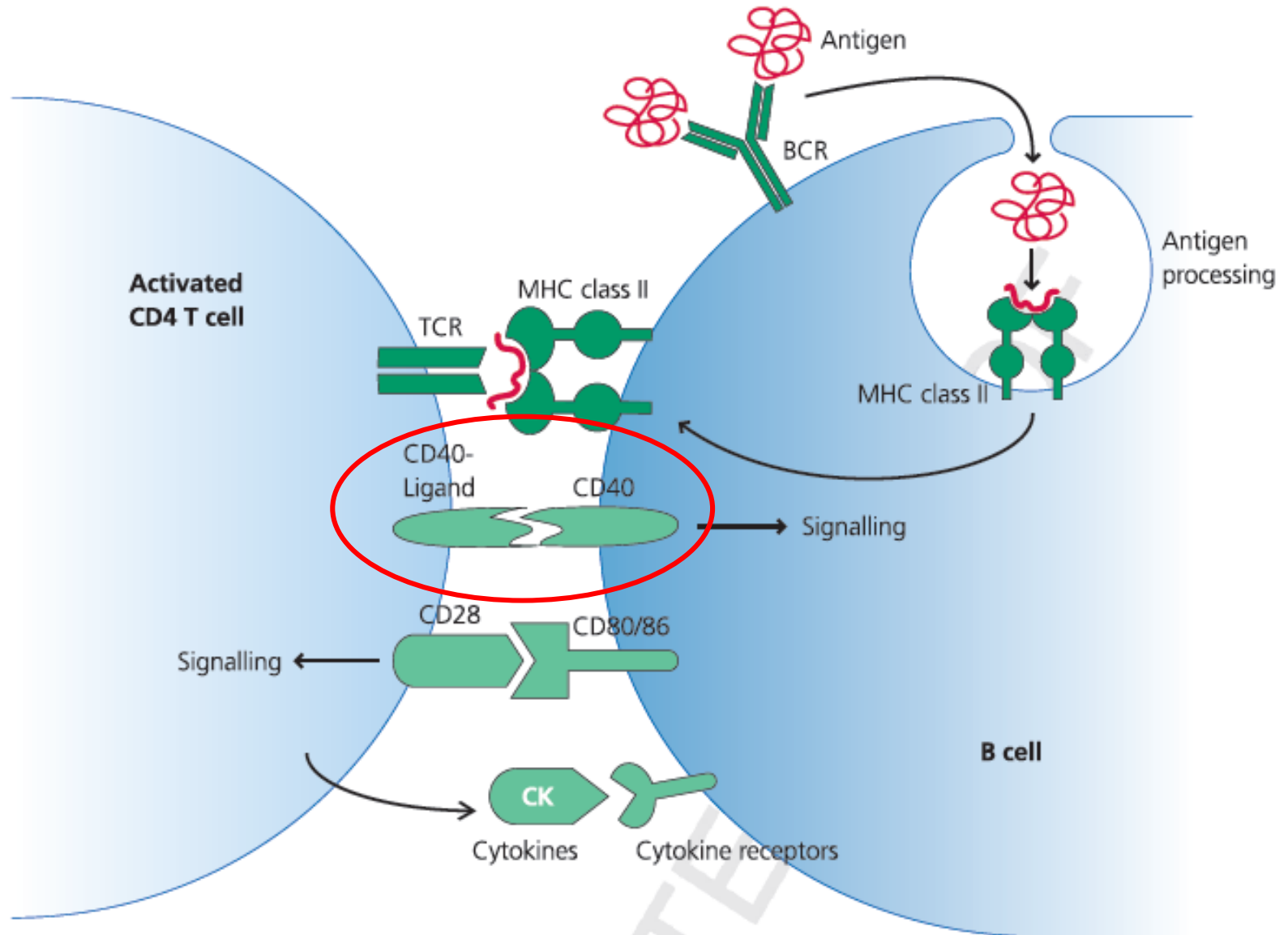
- Best response to microbes that activate complement.
- Complement coated microbes (C3dg) binds T cell by binding the slg and CD21.
- CD21 acts as complement receptor.
- The simultaneous binding of pathogen to both the co-receptor and BCR enhances the signal to the B cell.



Molecules Involved in T–B Cell Interactions

- T–B Cell Interaction is required in TD responses.
- B-cells act as APC that can present Ag for T-cells and activate them.
- They express MHCII molecules that present peptide antigens for T-cells.
- T cell activation requires two signals:
 - Signal 1: engagement of TCR with peptide-MHC complex.
 - Signal 2: provided by co-stimulatory molecules e.g. B7, CD40, ICOSL

T-B interactions



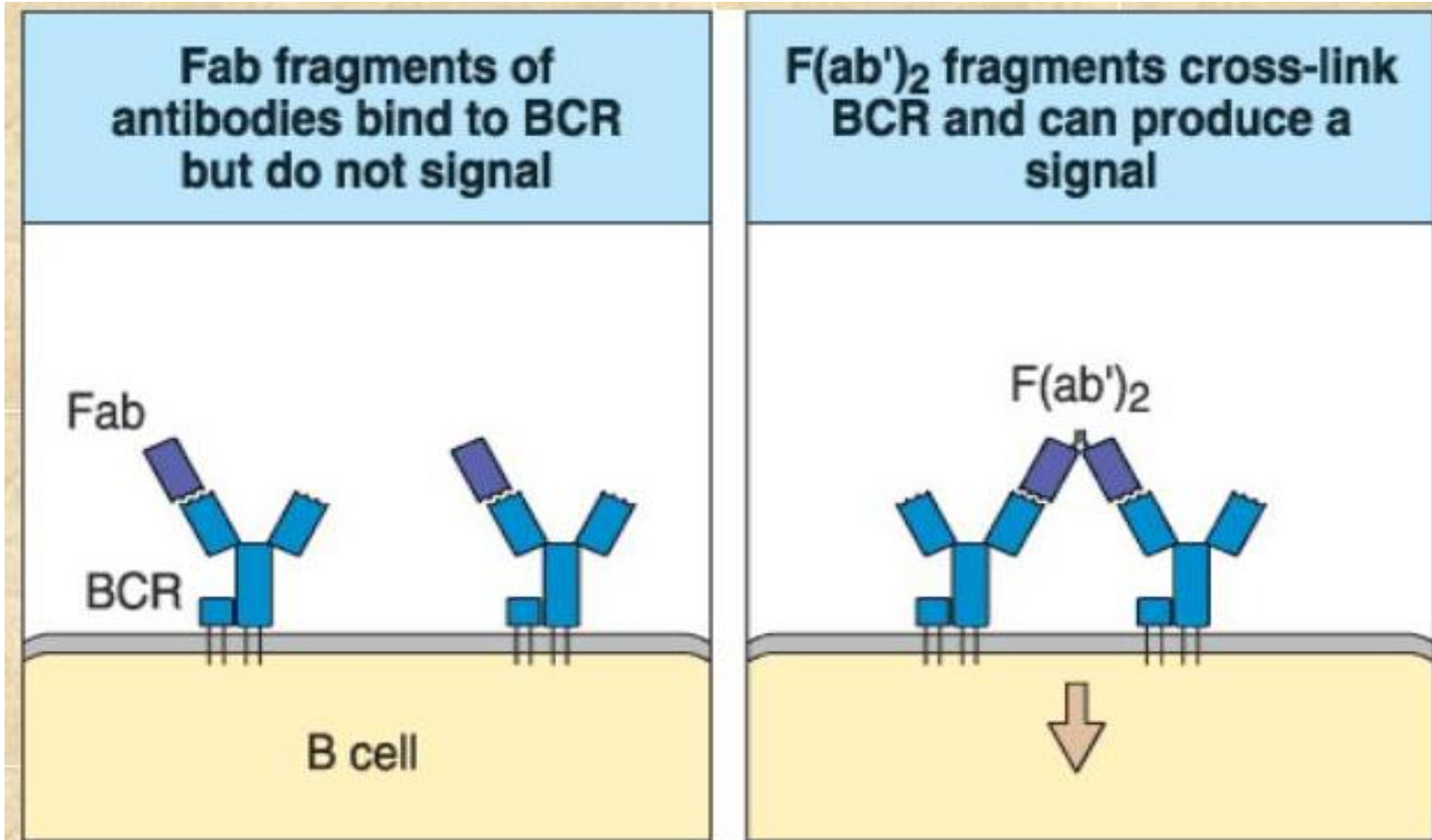
Signal transduction in B cells

- The recognition of antigen at the cell surface triggers multiple intracellular cascades that spread to the nucleus.
- The result is that B cell changes its pattern of gene expression.
- Activation of the cascade is initiated by ***receptor cross-linking*** — it brings together more than one BCR complex in the cell membrane.
- Antigen binding leads to phosphorylation of the ITAM part of Ig α /Ig β .
- This will bring and activate a series of intracellular kinases to the BCR complex. e.g. BTK

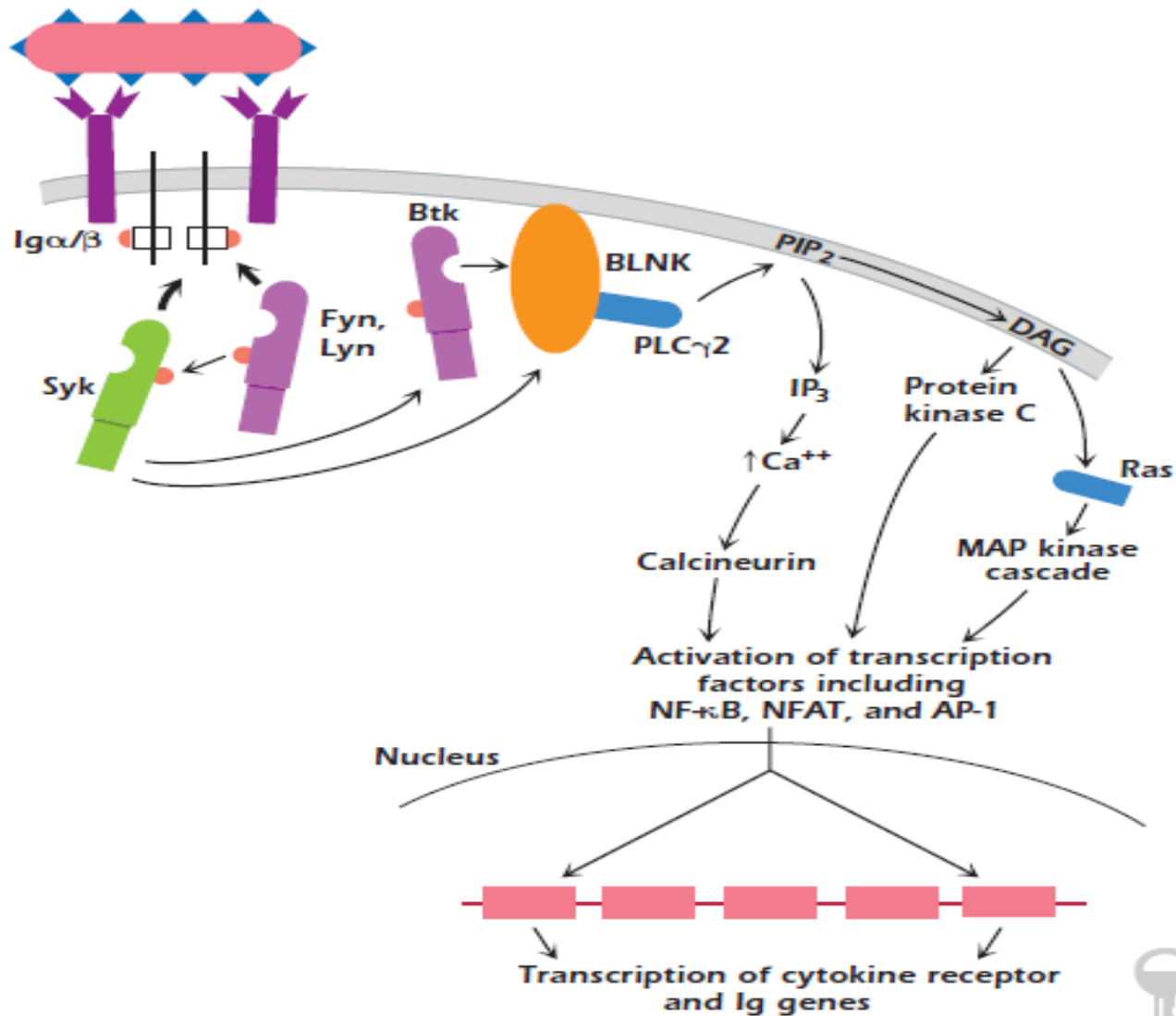
Signal transduction in B cells

- The recruitment, activation, and aggregation of these molecules result in the formation of a large signaling complex on the cytoplasmic side of the membrane associated with the BCR.
- This lead to the activation of transcription factors e.g. NF-AT, AP-1, and NF- κ B, that enter the nucleus and alter gene expression.
- The result is transcription of several genes involved in proliferation and the synthesis of Ig and cytokine receptors.
- The signal is enhanced by activation of the co-receptor.
- Negative regulation of the BCR signaling: CD22 (co-receptor), CD32-antibody feedback.

Receptor cross linking



BCR signaling

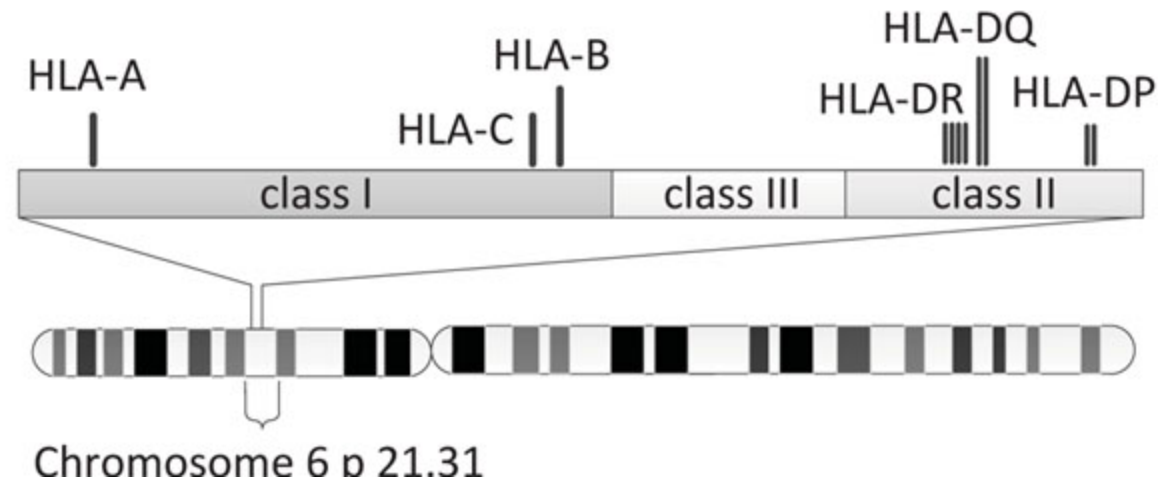


Major histocompatibility complex and Antigen processing and presentation

Dr. Dalia Wehedi

MHC molecules

- Major histocompatibility complex:
 - Cluster of genes found in all mammals
 - Its products play role in discriminating self/ non-self
 - Participants in both humoral and cell mediated immunity
- Acts as antigen presenting structure
- In humans- chromosomes 6 & referred to as HLA complex



MHC molecules

- The major histocompatibility complex (MHC) is a large genetic complex with multiple loci.
- Genes in the MHC were first identified as being important genes in rejection of transplanted tissues.
- There were three kinds of molecules encoded by the MHC “Class I, Class II and Class III”
- The major histocompatibility complex (MHC) products play roles in **intercellular recognition** and in discrimination between **self and non-self**

MHC molecules

- The MHC participates in the development of both **humoral** and **cell-mediated immune responses**.
- While antibodies may react with antigens alone, T cells recognize antigens **only** when it is combined with an MHC molecule.
- A peptide must associate with a given MHC of that individual, otherwise no immune response can occur.

MHC molecules

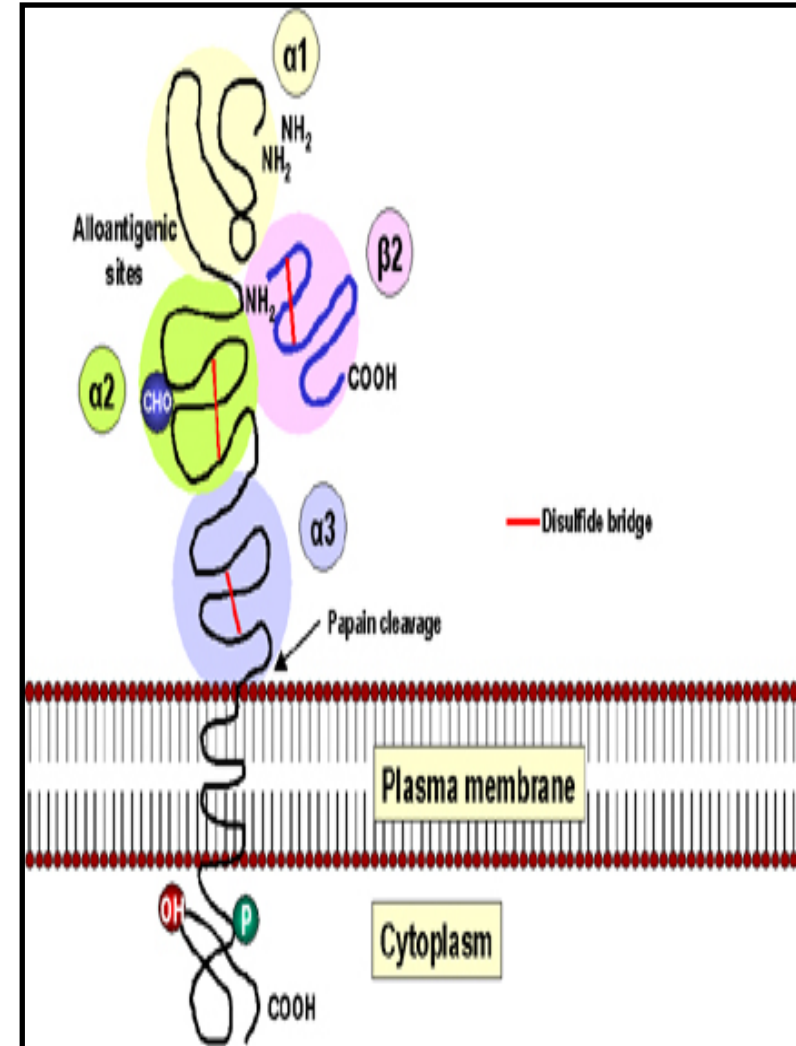
- MHC genes encode 3 classes of molecules:
 1. **Class I MHC genes** encode glycoproteins
- expressed on the surface of nearly all nucleated cells; but vary in concentration on different cell types
- Major function of Class I gene products is presenting peptide antigens to Cytotoxic T cells (CD8) cells.
- Class I antigens are produced by the A, B, and C subloci genes

MHC molecules

- **Class II MHC genes** encode glycoproteins
- expressed primarily on antigen-presenting cells: (macrophages, dendritic cells, and B cells), where they present processed antigenic peptides to TH cells.
- Class II antigens are produced by the DP, DQ, and DR subloci genes
- **Class III MHC genes** encode various secreted proteins that have
- Immune functions, including components of the complement system, and molecules involved in inflammation.

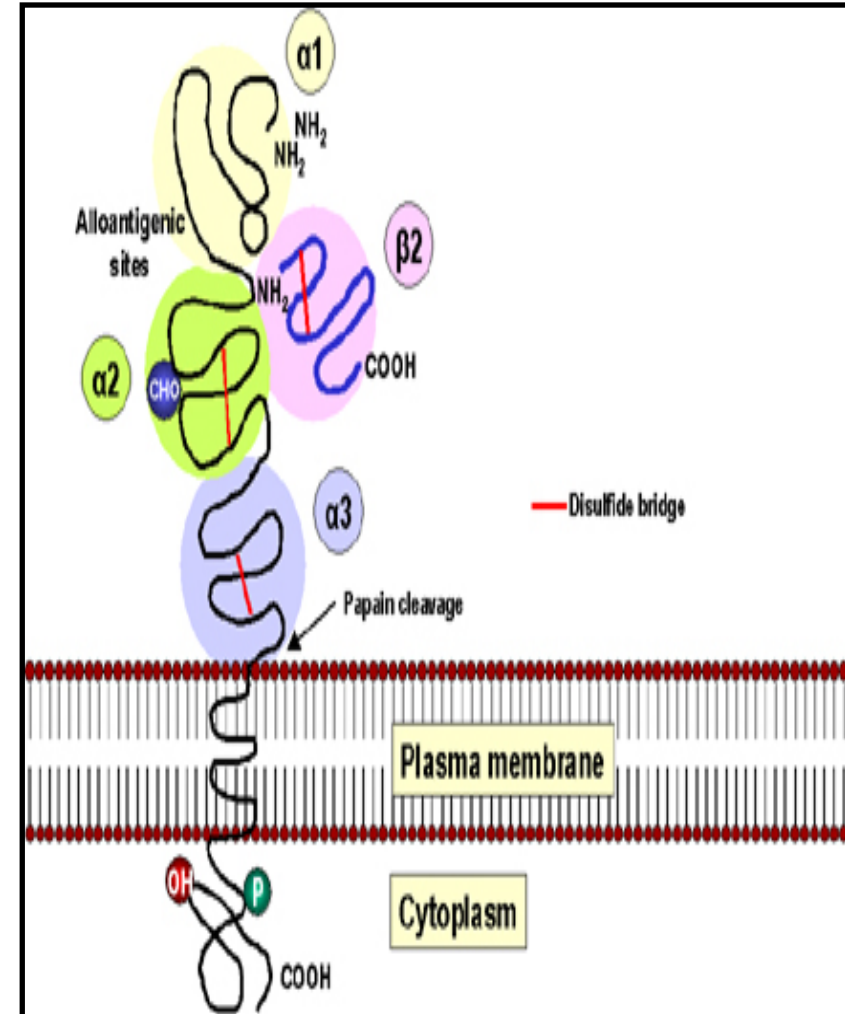
Structure of MHC I

- Two polypeptide chains, a long α chain and a short β ($\beta 2$ microglobulin)
- Four regions
 - Cytoplasmic region containing sites for phosphorylation and binding to cytoskeletal elements
 - Transmembrane region containing hydrophobic amino acids



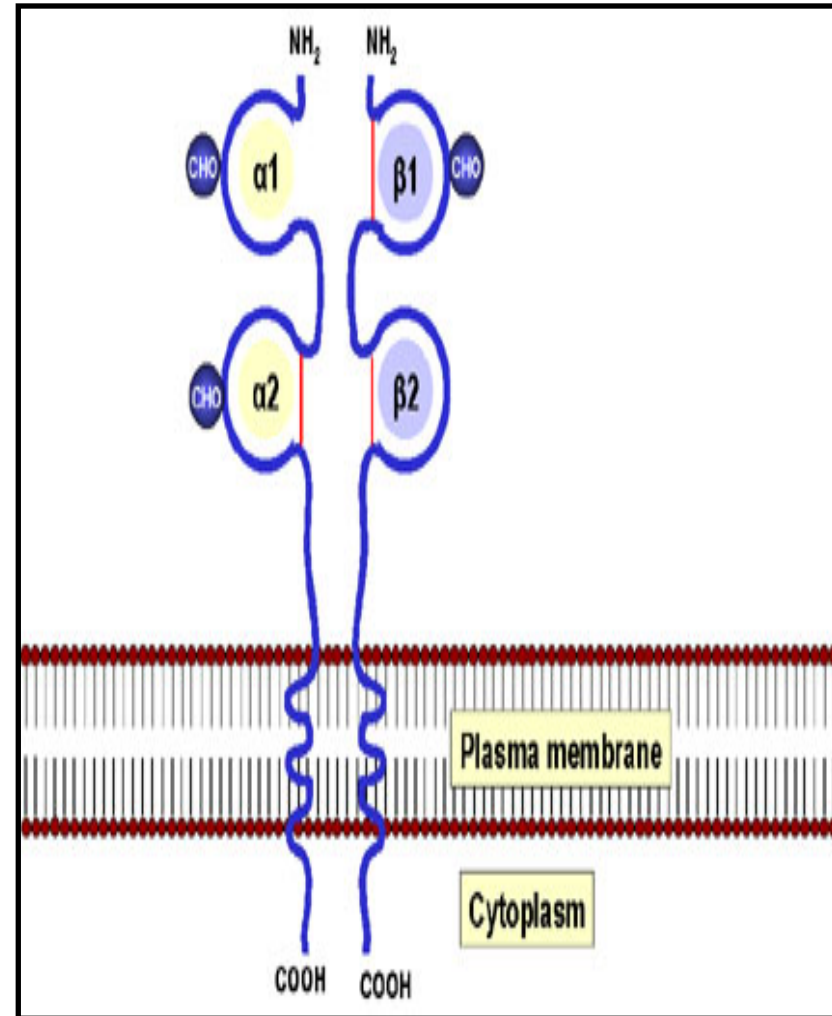
Structure of MHC I

- Four regions
 - A highly conserved $\alpha 3$ domain to which CD8 binds
 - A highly polymorphic peptide binding region formed from the $\alpha 1$ and $\alpha 2$ domains
- B2-microglobulin helps stabilize the conformation



Structure of MHC II

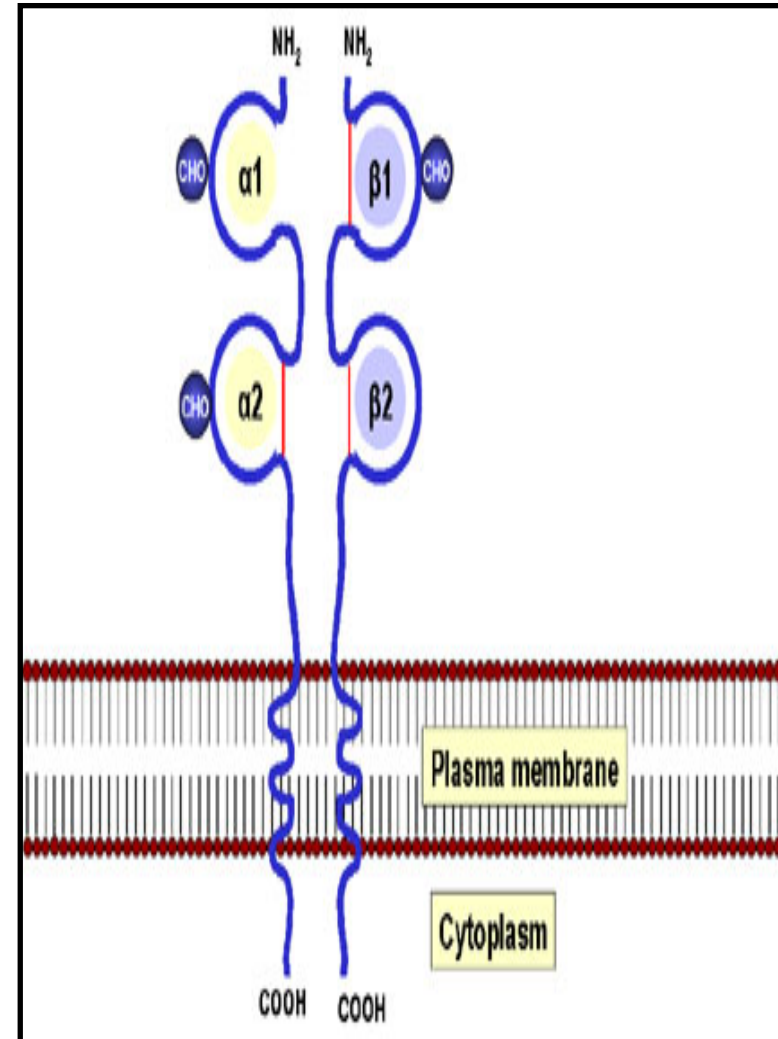
- Two polypeptide chains, α , and β , of roughly equal length
- Four regions
 - Cytoplasmic region containing sites for phosphorylation and binding to cytoskeletal elements



Structure of MHC II

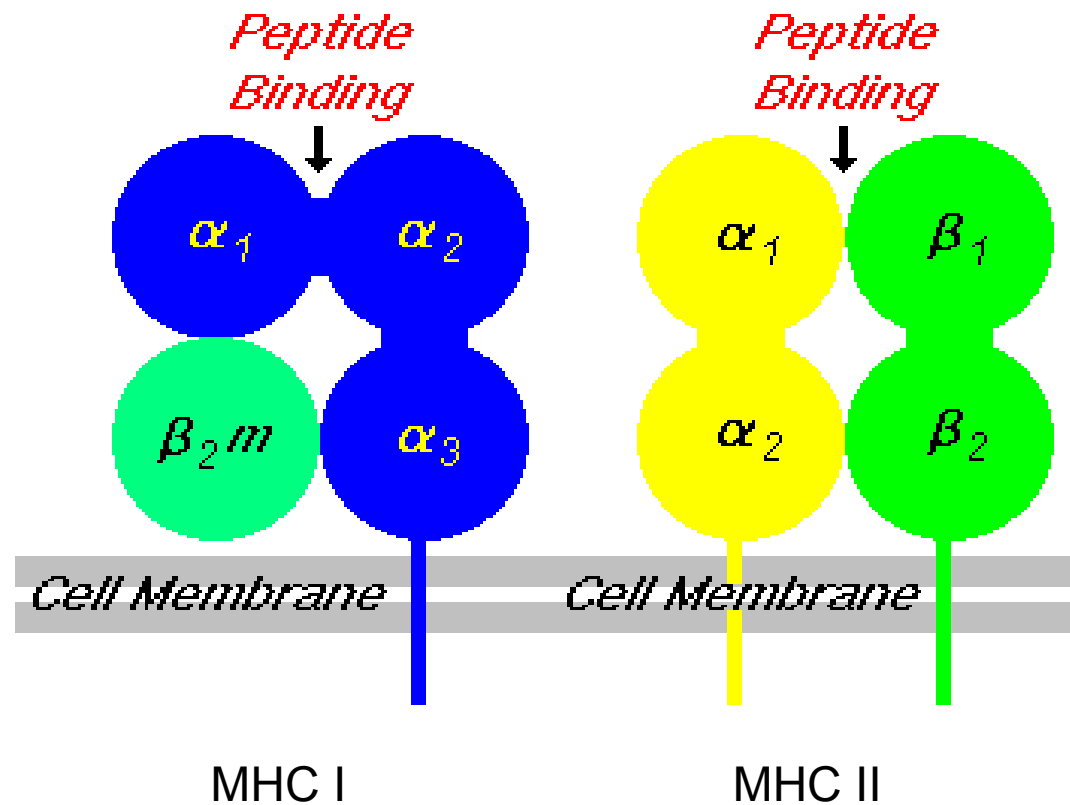
Four regions

- Transmembrane region containing hydrophobic amino acids
- A highly conserved $\alpha 2$ and a highly conserved $\beta 2$ domains to which CD4 binds
- A highly polymorphic peptide binding region formed from the $\alpha 1$ and $\beta 1$ domains

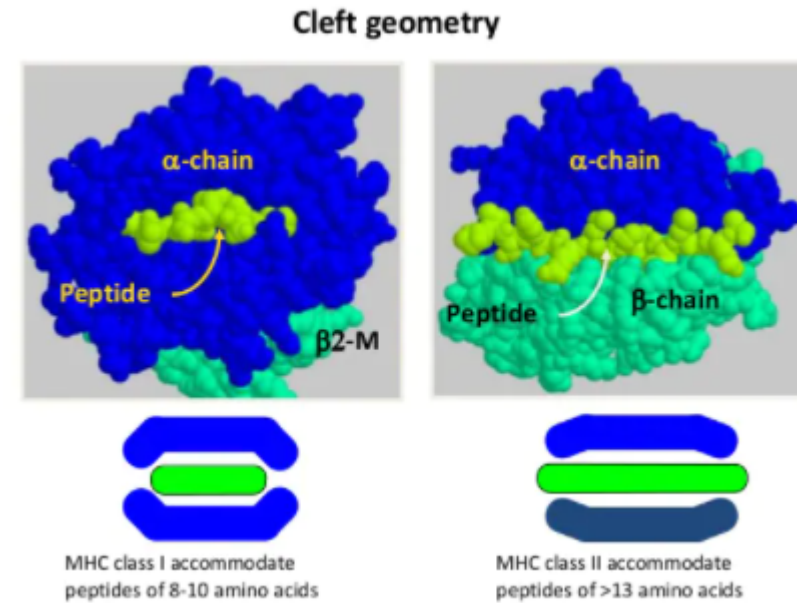
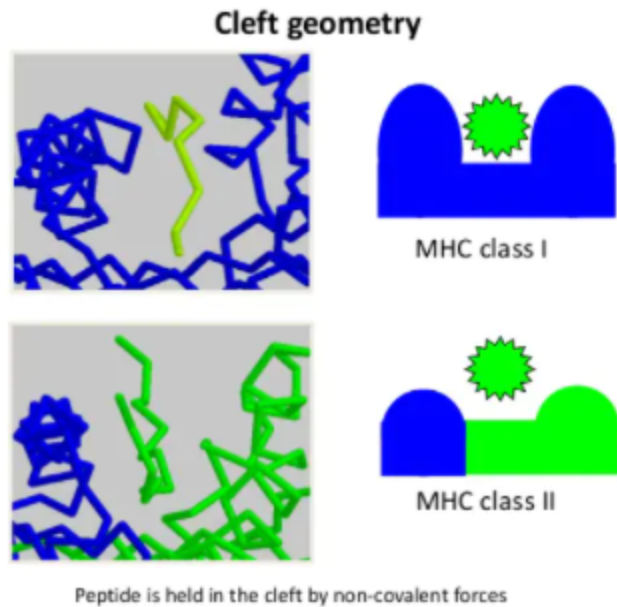


Structure of MHC

MHC I Compared to MHC II Structure



Cleft geometry



Peptide binding by class I and class II MHC molecules

	Class I molecule	Class II molecule
Peptide binding domain		
Nature of peptide binding cleft	Closed at both ends	Opened at both ends
General size of bound peptide	8-10 amino acids	13-18 amino acids
Peptide motifs involved in binding to MHC molecules	Anchors the residues at both ends of peptide	Anchors the residues distributed along the length of the peptide
Nature of bound peptide	Extended structure in which both ends interact with MHC cleft but middle arches up away from the MHC molecule	Extended structure that is held at a constant elevation above the floor of the MHC cleft

Cellular Distribution of MHC Molecules

- In general, the classical class I MHC molecules are expressed on **most nucleated cells**, but the level of expression differs among different cell types.
- The highest levels of class I molecules are expressed by lymphocytes
- In contrast, fibroblasts, muscle cells, liver hepatocytes, and neural cells express very low levels of class I MHC molecules.

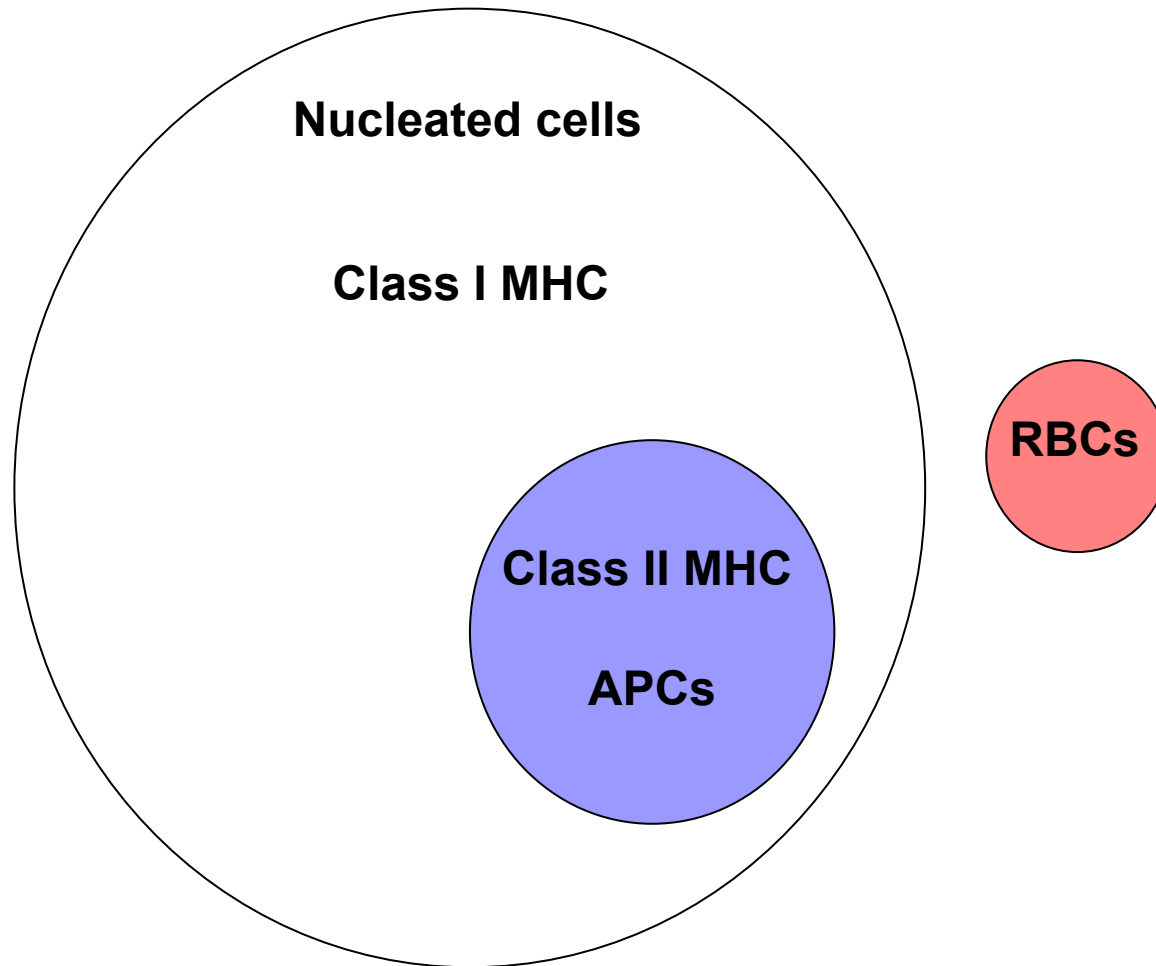
Cellular Distribution of MHC Molecules

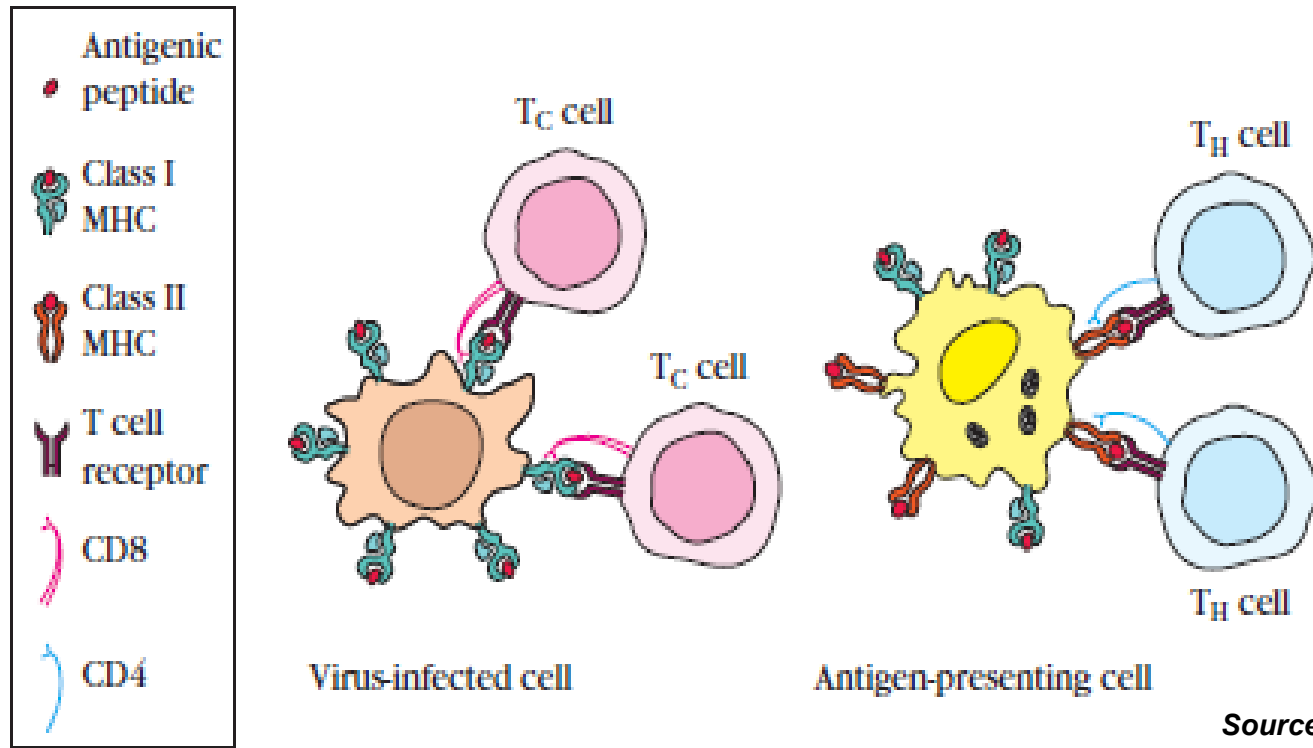
- The low level on liver cells may contribute to the considerable success of liver transplants by reducing the likelihood of graft recognition by Tc of the recipient
- In normal, healthy cells, the class I molecules will display self-peptides resulting from normal turnover of self proteins.
- In cells infected by a virus, viral peptides, as well as self peptides, will be displayed

Cellular Distribution of MHC Molecules

- Unlike class I MHC molecules, class II molecules are expressed only by
 - Antigen-presenting cells, primarily macrophages, dendritic cells, and B cells.
- Among the various cell types that express class II MHC molecules, marked differences in expression have been observed, depending on maturation and/or degree of antigenic stimulation.

Cellular Distribution of MHC Molecules





Class I MHC molecules are expressed on nearly all nucleated cells. Class II MHC molecules are expressed only on antigen-presenting cells. T cells that recognize only antigenic peptides displayed with a class II MHC molecule generally function as T helper (T_H) cells. T cells that recognize only antigenic peptides displayed with a class I MHC molecule generally function as T cytotoxic (T_C) cells.

Regulation of MHC Expression

- The expression of MHC molecules is regulated by various cytokines.
 - interferons (alpha, beta, and gamma) and tumor necrosis factor increase the expression of class I MHC molecules on cells.
 - Interferon gamma (IFN- gamma), for example, appears to induce the formation of a specific transcription factor to induce the expression of class I MHC genes.

Regulation of MHC Expression

- MHC molecules express major self antigens.
- The MHC partly determines the ability of an individual's Tc and Th cells' response to antigens of infectious organisms,
 - It has therefore been implicated in the susceptibility to disease, and
 - In the development of autoimmunity.

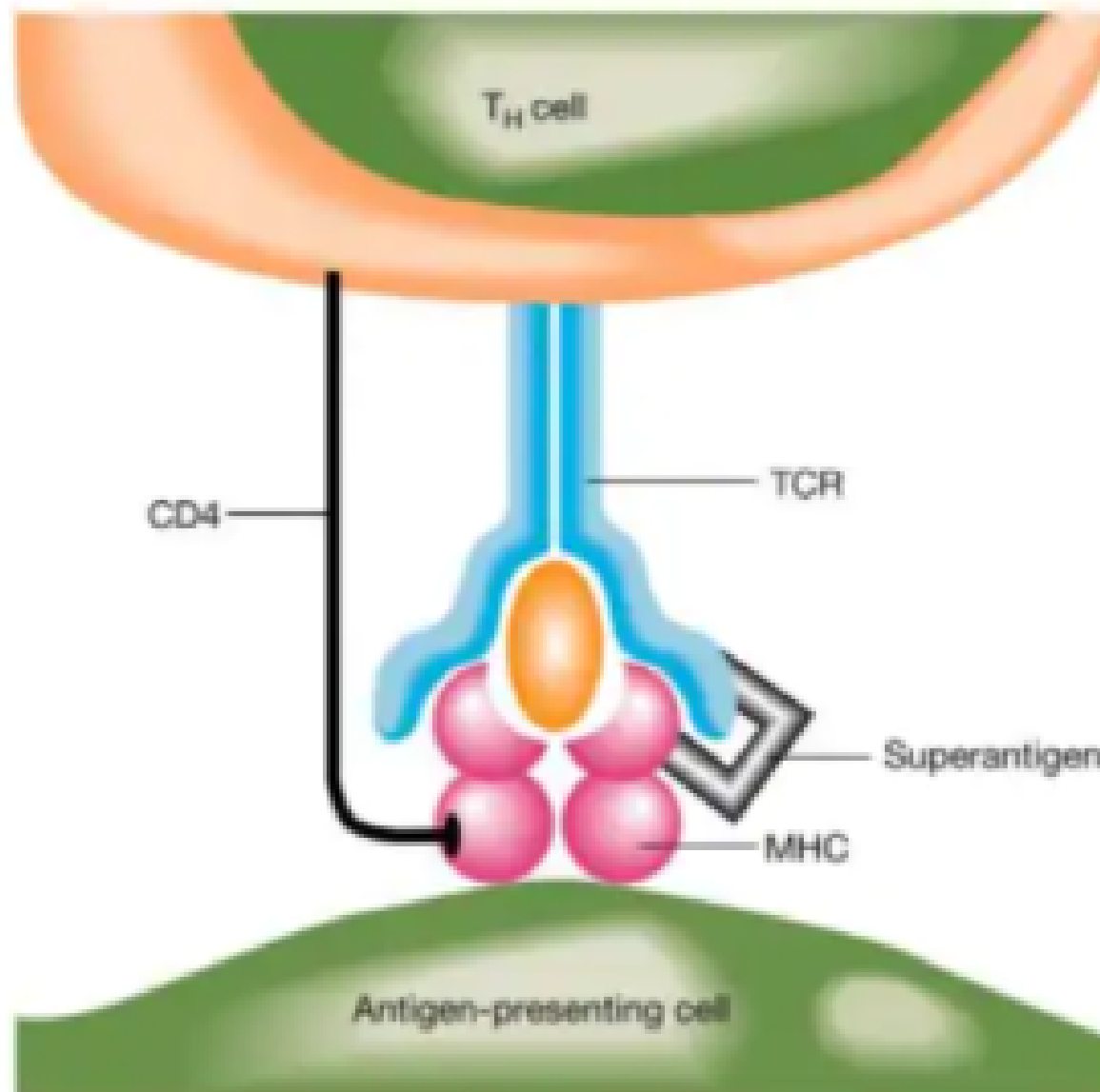
Regulation of MHC Expression

- Other cytokines influence MHC expression only in certain cell types; for example,
 - IL-4 increases expression of class II molecules by resting B cells.
 - Expression of class II molecules by B cells is down-regulated by IFN- γ
 - MHC expression is decreased by infection with certain viruses, including human cytomegalovirus (CMV), hepatitis B virus (HBV), and adenovirus 12 (Ad12).

MHC Molecules and Immune Responsiveness

- Immune responsiveness's dependence on the class II MHC reflects the importance of class II MHC molecules in presenting antigen to TH cells.
- Absence of an MHC molecule that can bind and present a given peptide, or the absence of T-cell receptors that can recognize a given peptide–MHC molecule complex,
 - May result in the absence of immune responsiveness.

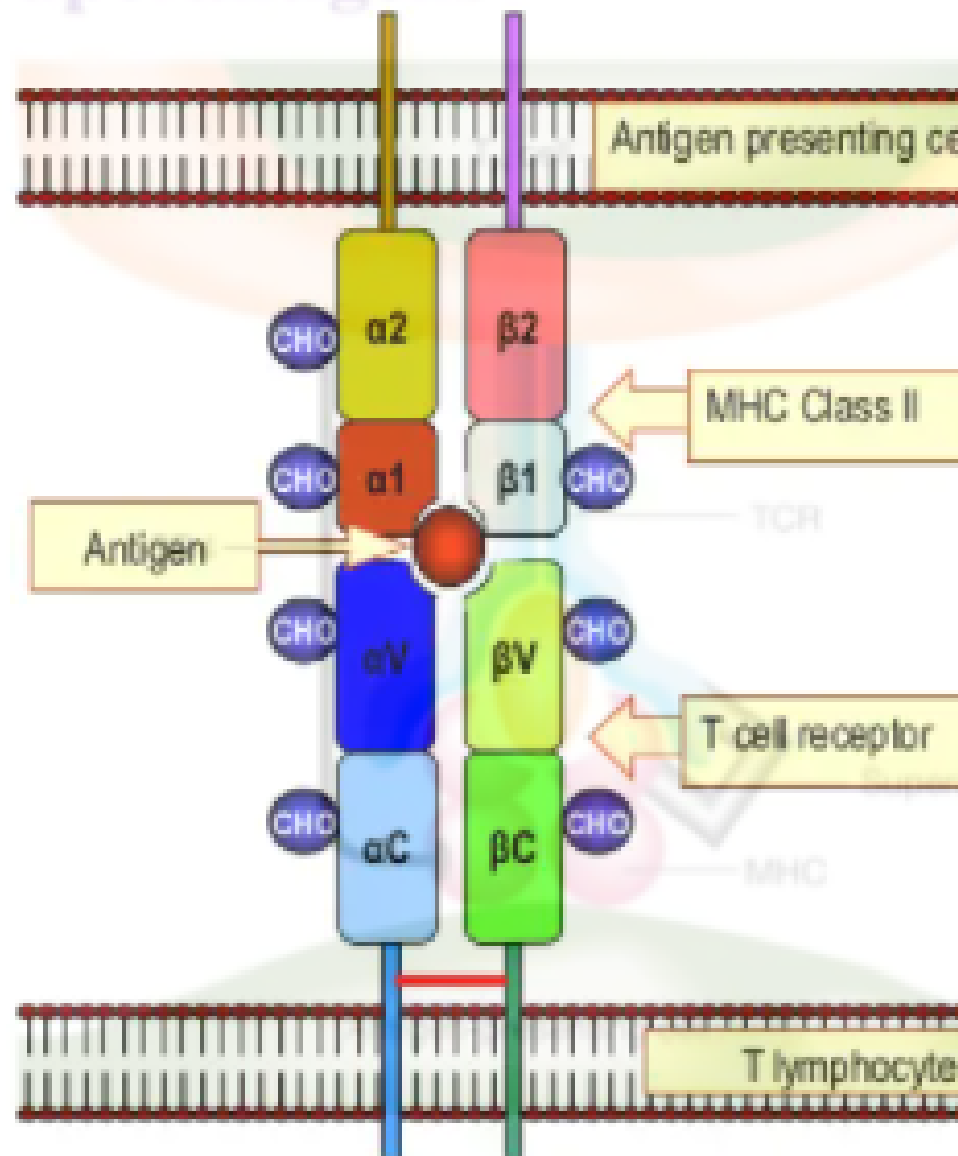
Superantigens



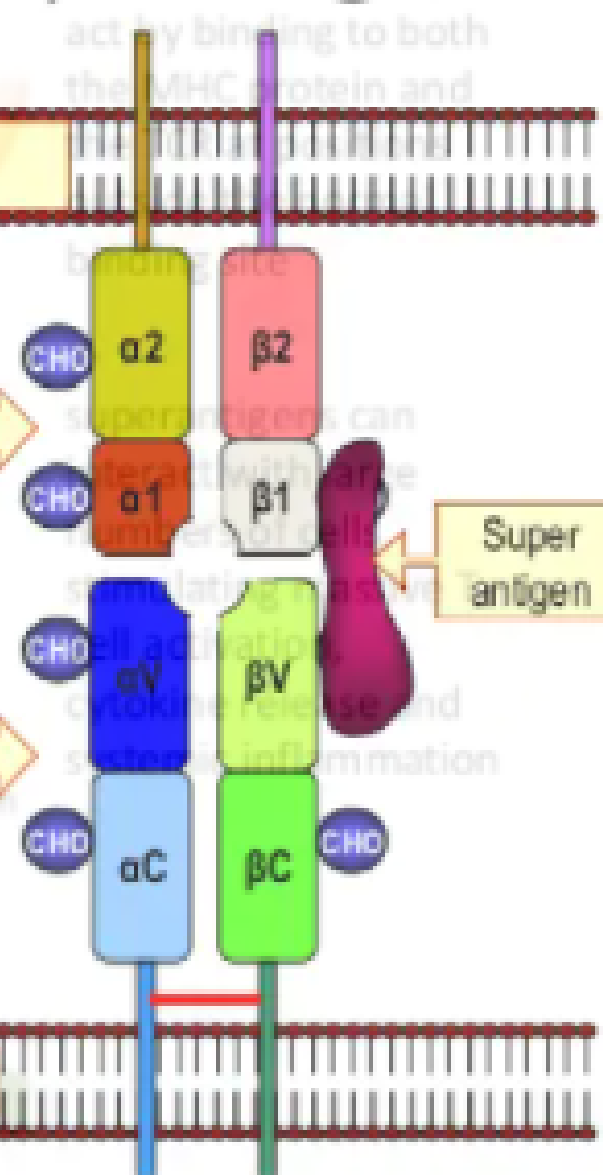
Bacterial superantigens act by binding to both the MHC protein and the TCR at positions outside the normal binding site

superantigens can interact with large numbers of cells, stimulating massive T-cell activation, cytokine release and systemic inflammation

Conventional Antigen



Superantigen



Superantigens

- Conventional antigens are only recognized by specific T4-cells having a TCR with a corresponding shape.
- Superantigens are unusual bacterial toxins that interact with exceedingly large numbers of T4-lymphocytes.
- Activation of very large numbers of T4-lymphocytes results in the secretion of excessive amounts of a cytokine called interleukin-2 (IL-2).
- Excess stimulation of IL-2 secretion can also lead to production of inflammatory and can lead to the same endothelial damage, acute respiratory distress syndrome, disseminated intravascular coagulation, shock, and multiple organ system failure
- Examples of superantigens include toxic shock syndrome toxin-1 (TSST-1), Streptococcal pyrogenic exotoxins (SPE), Staphylococcal enterotoxins (SE), and enterotoxigenic E. coli (ETEC) enterotoxin.

Antigen processing and presentation

MHC-restricted antigen recognition by T cells

- Any T cell can recognize an antigen on an APC only if that antigen is displayed by MHC molecules
- Antigen receptors of T cells have dual specificities:
 1. For peptide antigen (responsible for specificity of the immune response)
 2. For MHC molecules (responsible for MHC restriction)
- During maturation in the thymus, T cells whose antigens receptors see MHC are selected to survive and mature; therefore mature T cells are “MHC restricted”

Antigen processing

- Conversion of native antigen (large globular protein) into peptides capable of binding to MHC molecules.
- Occurs in cellular compartments where MHC molecules are synthesized and assembled (ER)
 - Determines how antigen in different cellular compartments generates peptides that are displayed by class I or class II molecules.

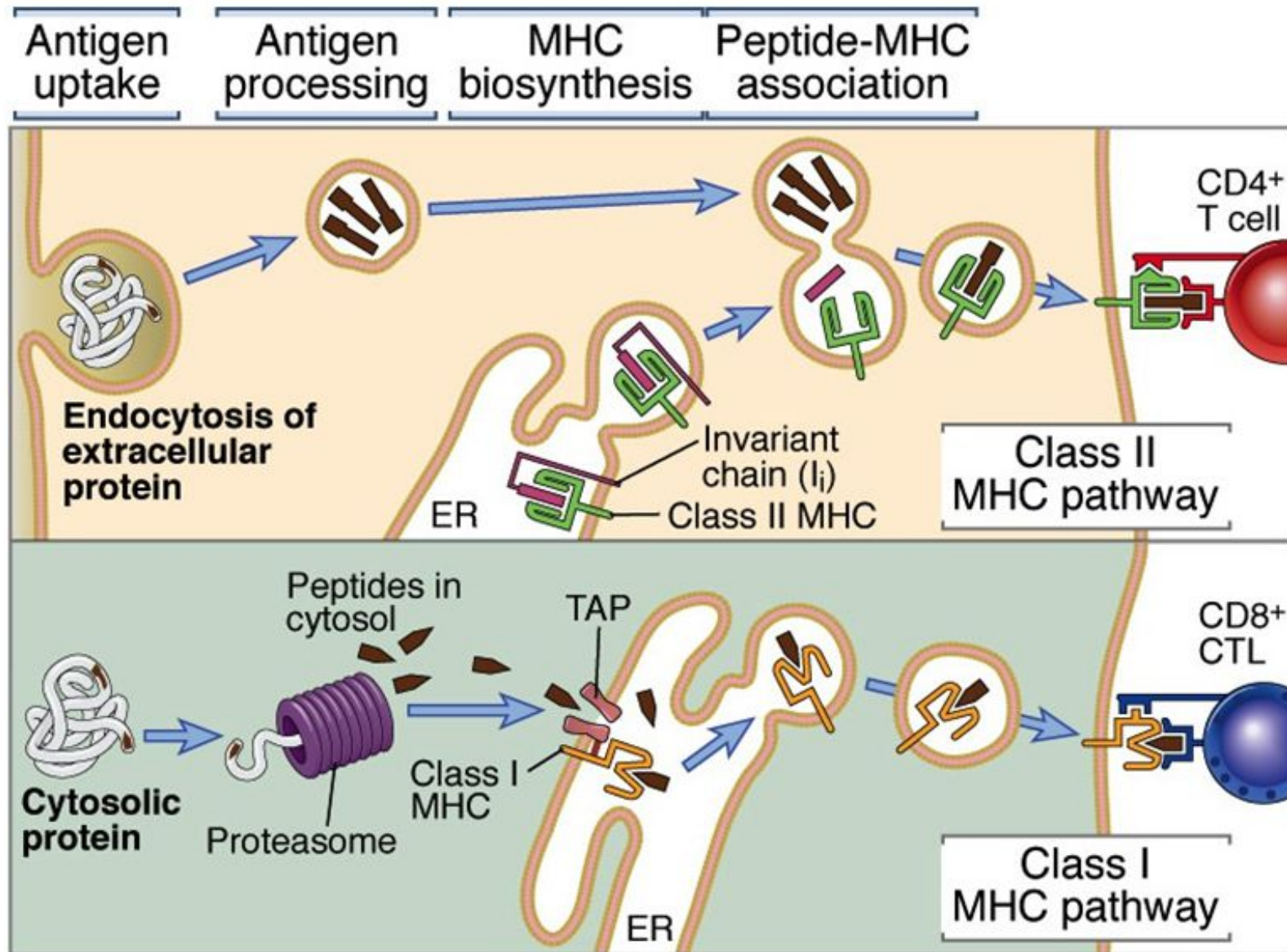
What do the $\alpha\beta$ T cell receptors (TCR) recognize?

1. Only fragments of proteins (peptides) associated with MHC molecules on surface of cells
2. Helper T cells CD4⁺ (Th) recognize peptide associated with MHC class II molecules
3. Cytotoxic T cells CD8⁺ (Tcyt) recognize peptide associated with MHC I molecules

Antigen presenting cells

Professional Antigen presenting cells	Non-professional Antigen presenting cells
Dendritic cells Macrophages B cells	Fibroblast (skin)
	Glial cells (Brain)
	Pancreatic beta cells
	Thymic epithelial cells
	Thyroid epithelial cells
	Vascular epithelial cells

Pathways of antigen processing



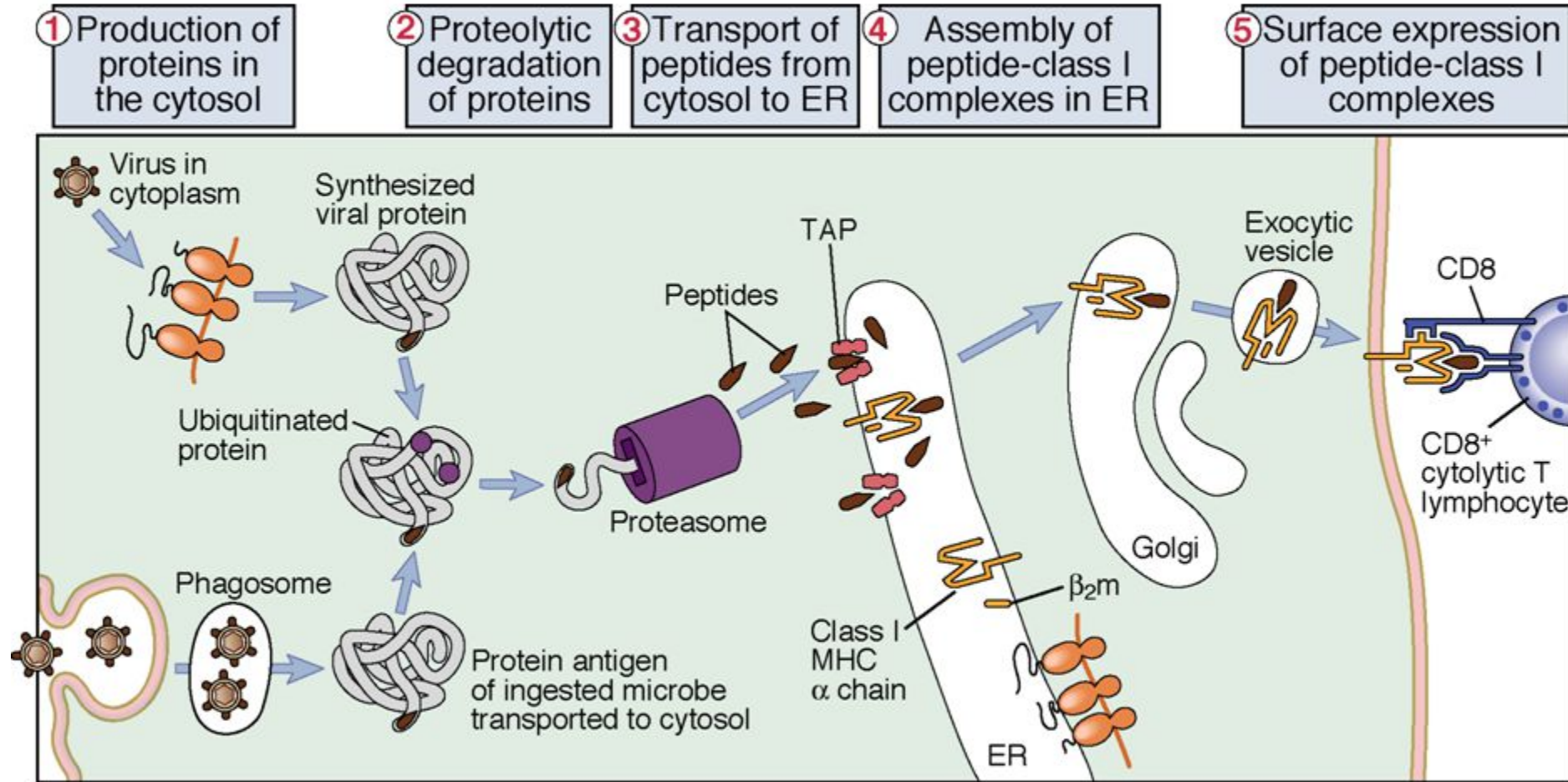
Protein antigen in vesicles --> class II MHC pathway

Protein antigen in cytosol (cytoplasm) --> class I MHC pathway

Class I MHC molecules (Endogenous Antigens: The cytosolic pathway)

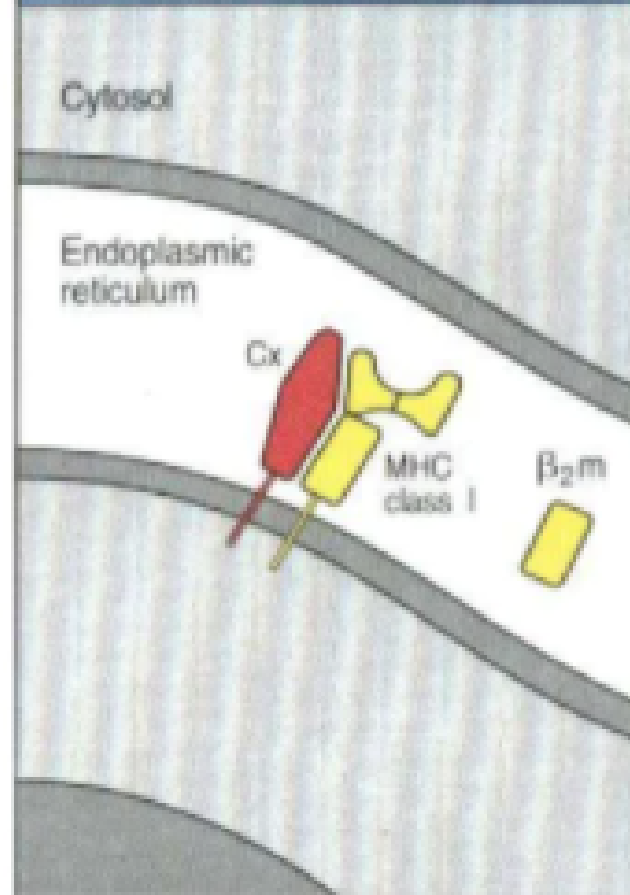
- Cytotoxic T cells need to kill cells containing cytoplasmic microbes, and tumor cells (which contain tumor antigens in the cytoplasm)
- Cytosolic proteins are processed into peptides that are presented in association with class I molecules
- Most cytosolic peptides derived from endogenously synthesized peptides (viral, tumor, ...) proteins
- All nucleated cells (which are capable of being infected with viruses or transformed) express class I.

The class I MHC pathway of processing of endogenous cytosolic protein antigens

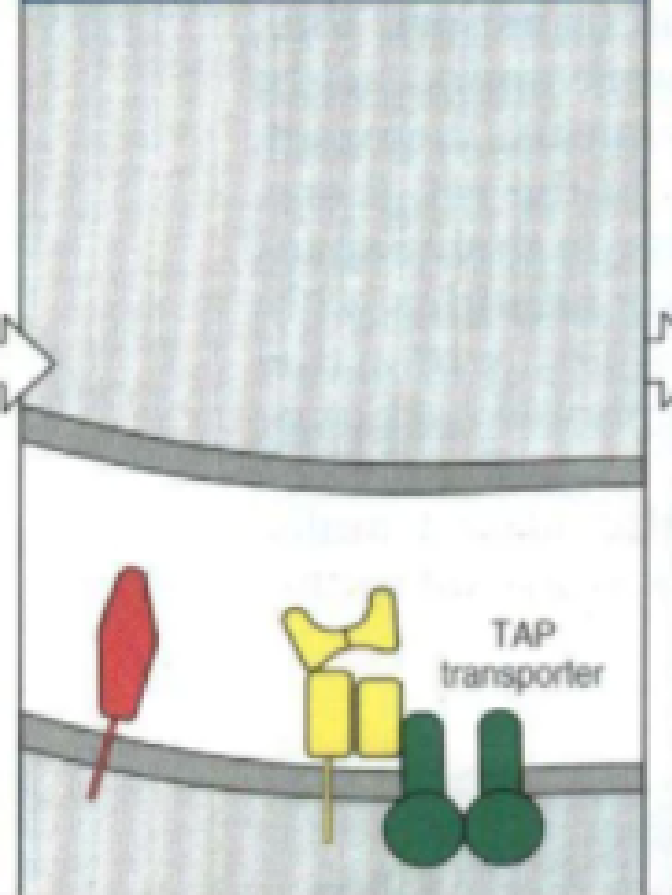


*Cytoplasmic peptides are actively transported into the ER;
class I MHC molecules are available to bind peptides in the ER*

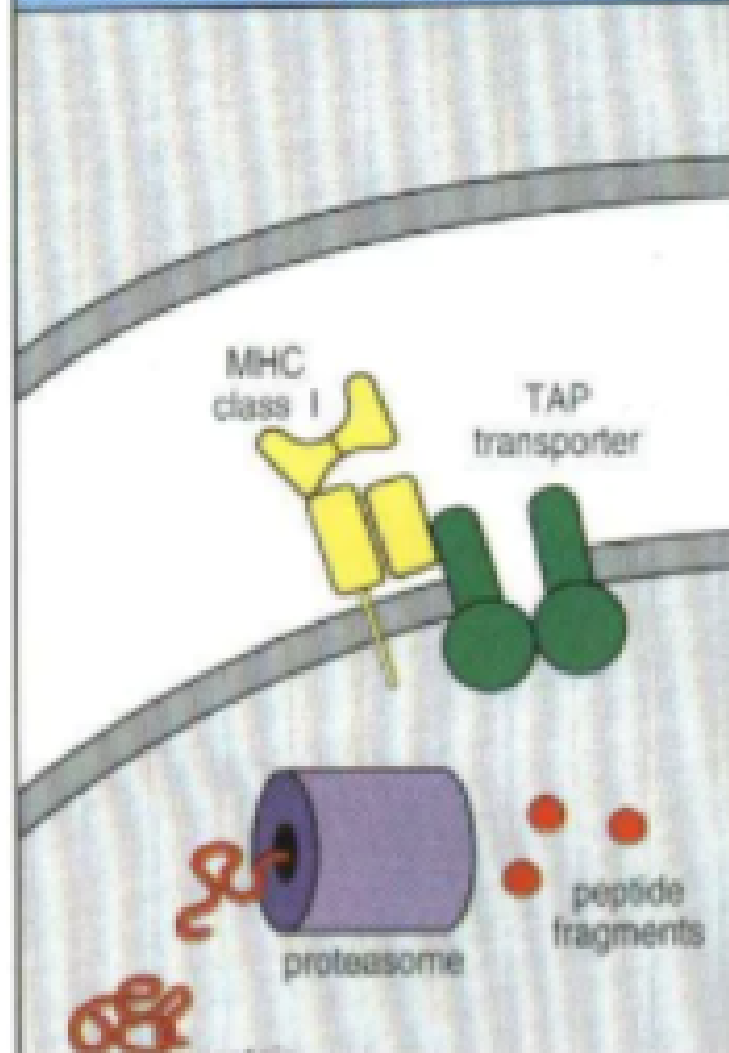
Partially folded MHC class I
 α chain binds to
calnexin (Cx)



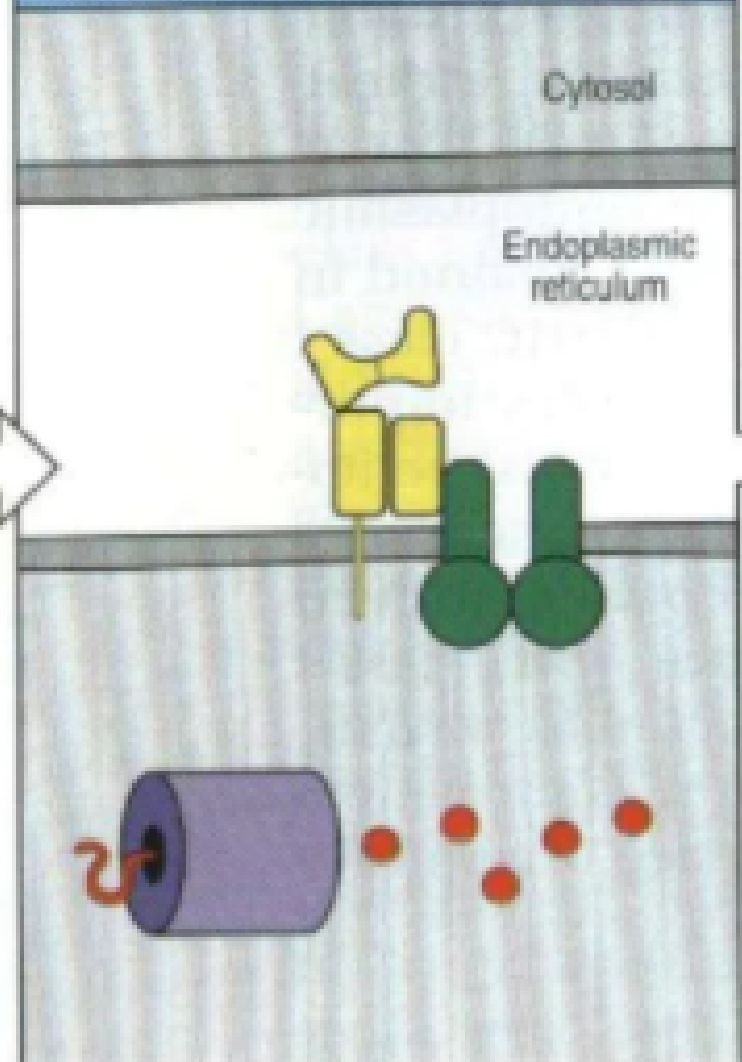
β_2 -microglobulin (β_2m) binds
partially folded MHC class I
molecule, which is released from
calnexin and binds to TAP-1



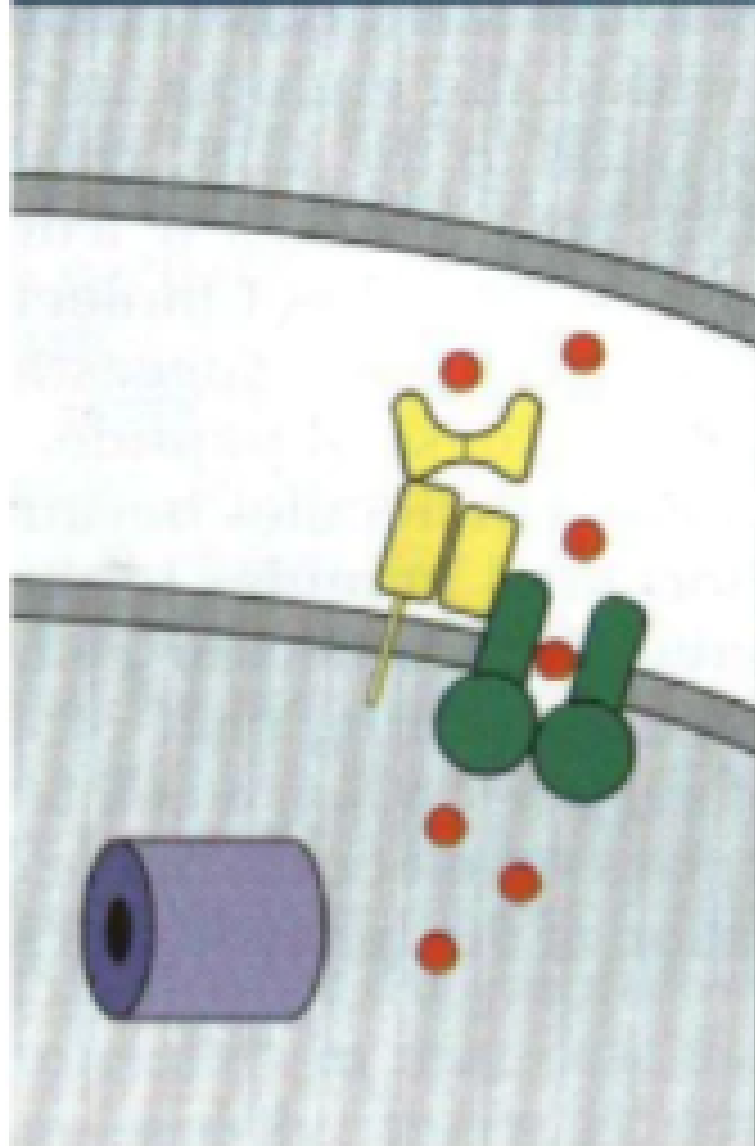
Cytosolic proteins are degraded to peptide fragments by the proteasome, a large multicatalytic protease



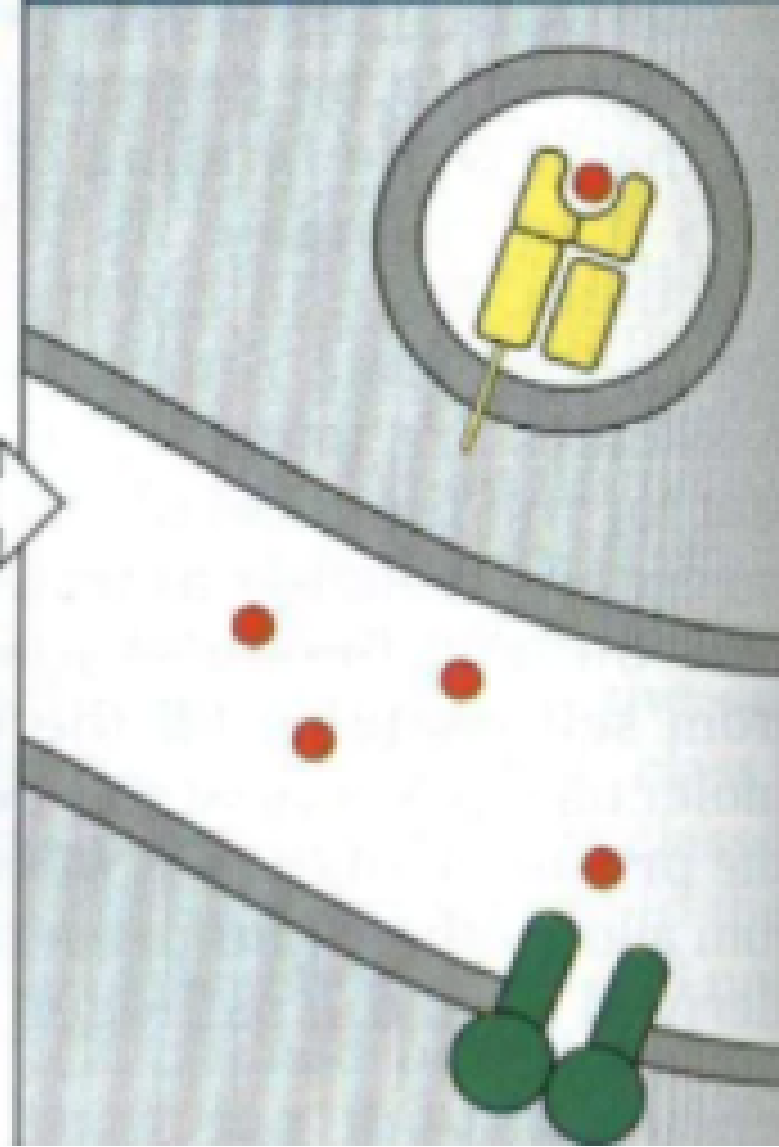
Peptides produced are inaccessible to the MHC class I molecule, which remains bound to TAP-1



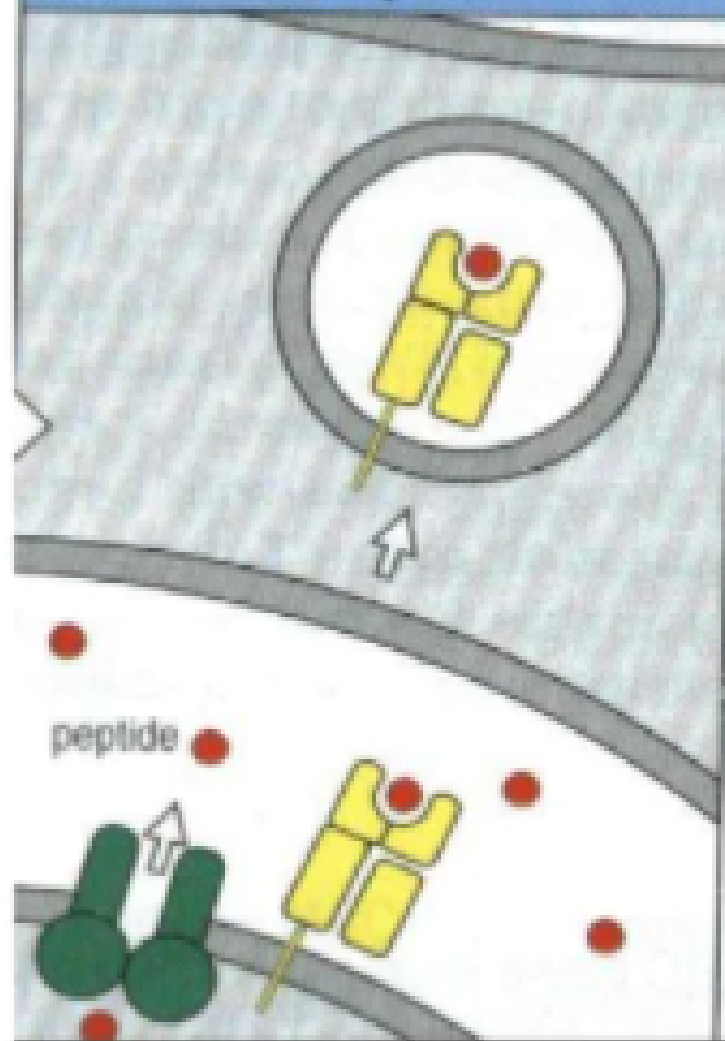
Peptides are transported into the lumen of the ER by the TAP transporter and are 'inspected' by the TAP-bound MHC class I



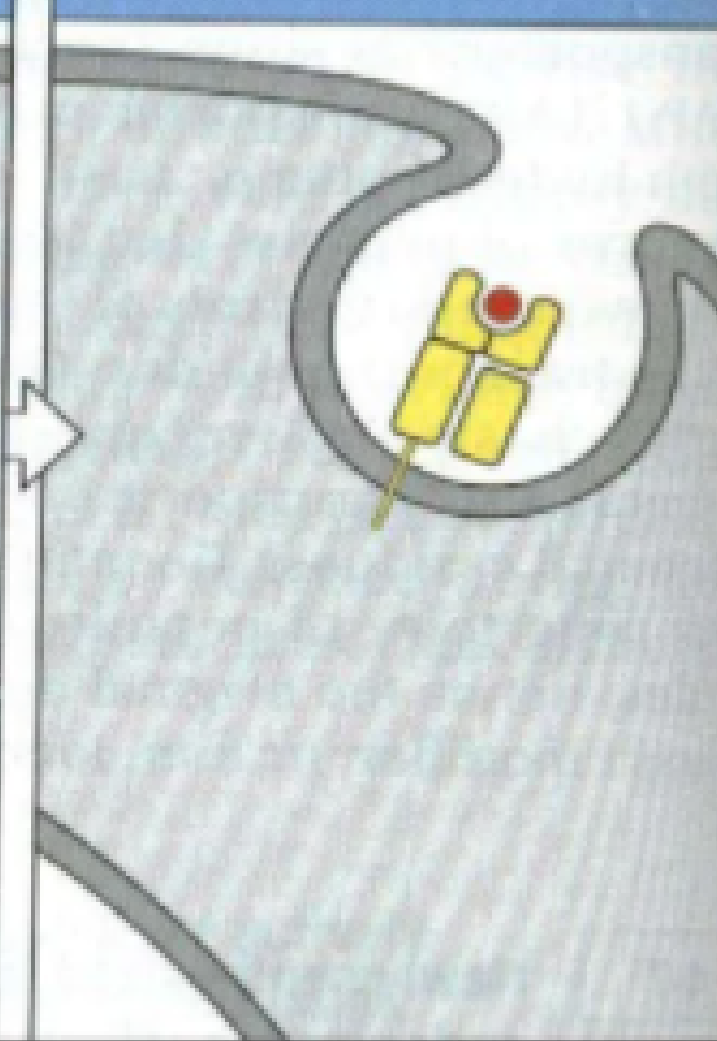
When a peptide binds MHC class I tightly, the MHC molecule folds around the peptide and is released from TAP-1

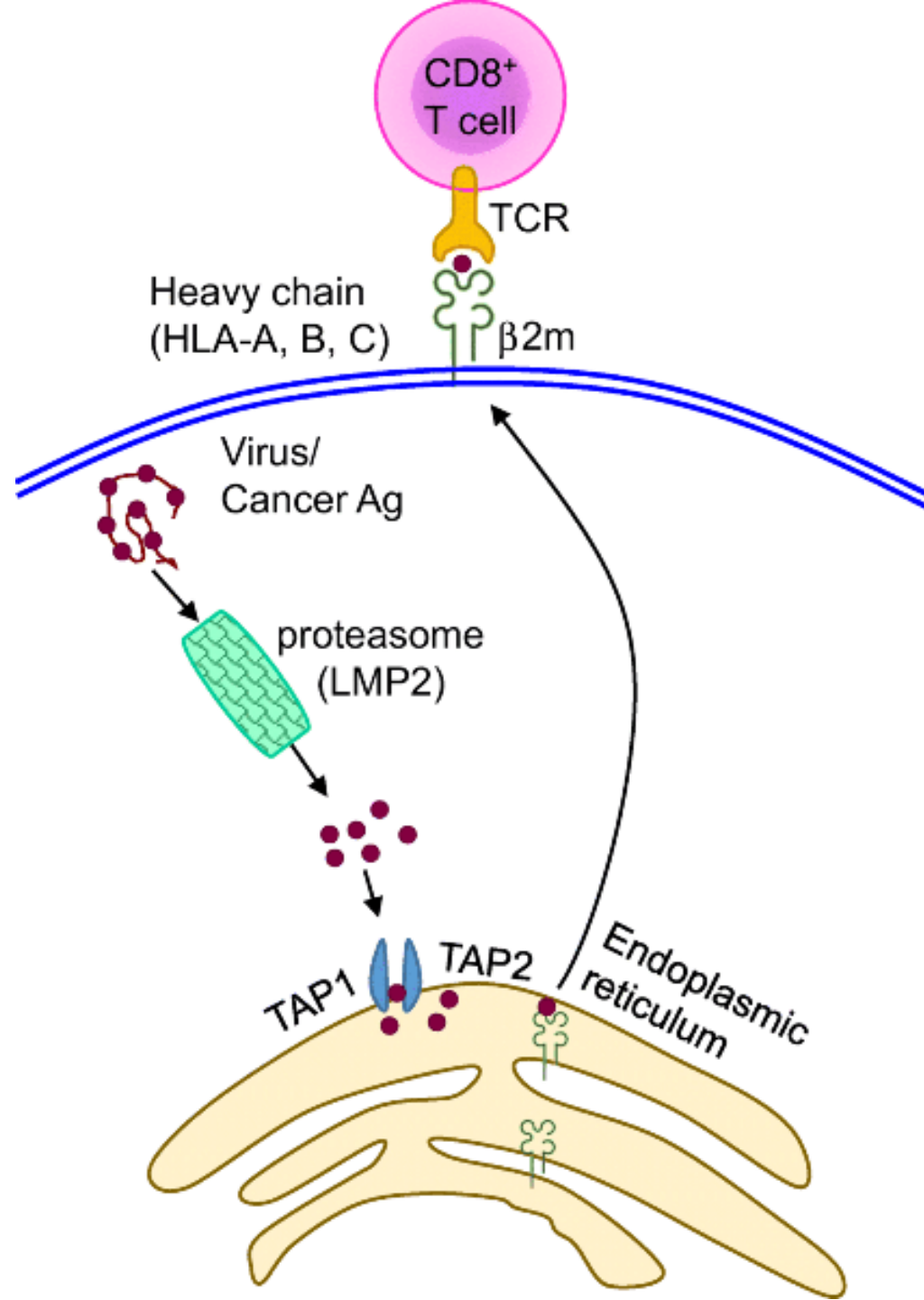


TAP transporter delivers a peptide that binds an MHC class I molecule and completes its folding. Fully folded MHC class I molecules are released from TAP-1 and exported



MHC class I molecule with bound peptide is delivered to the cell surface



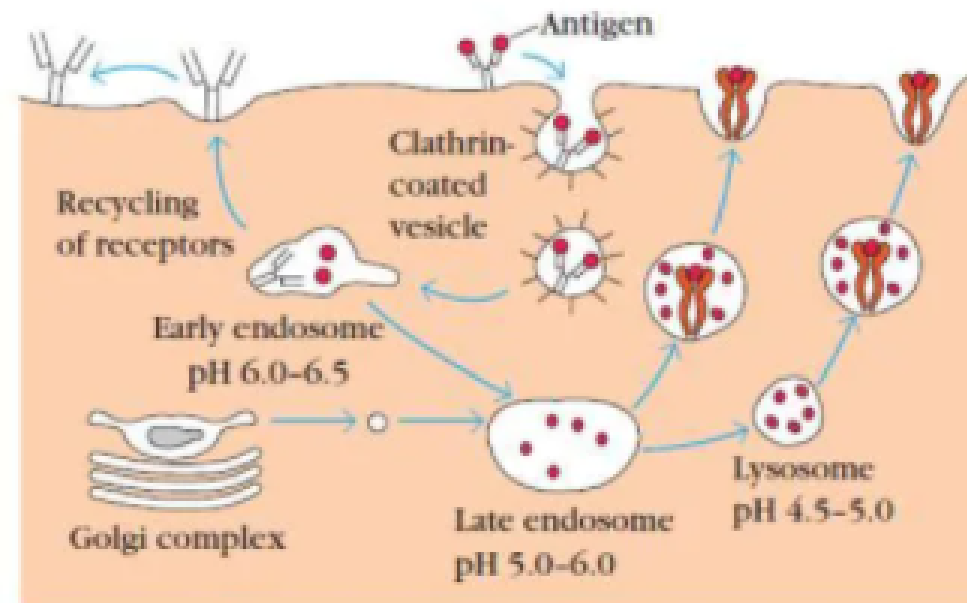


Exogenous pathogen: The endocytic pathway

Loading of antigen to MHC class II

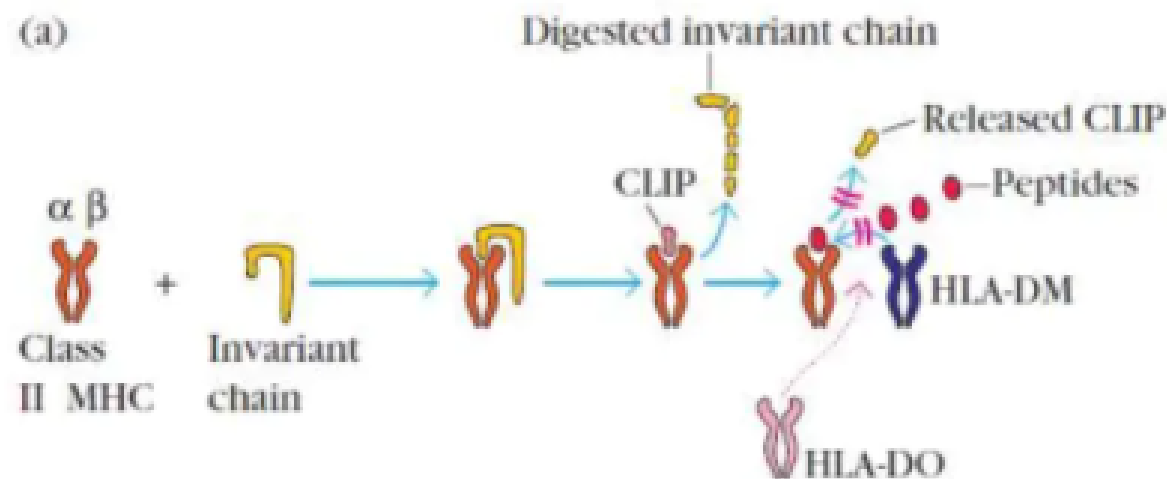
Generation of antigenic peptides in the endocytic processing pathway.

- Internalized exogenous antigen moves through several acidic compartments, in which it is degraded into peptides that ultimately associate with class II MHC molecules transported in vesicles from the Golgi complex.

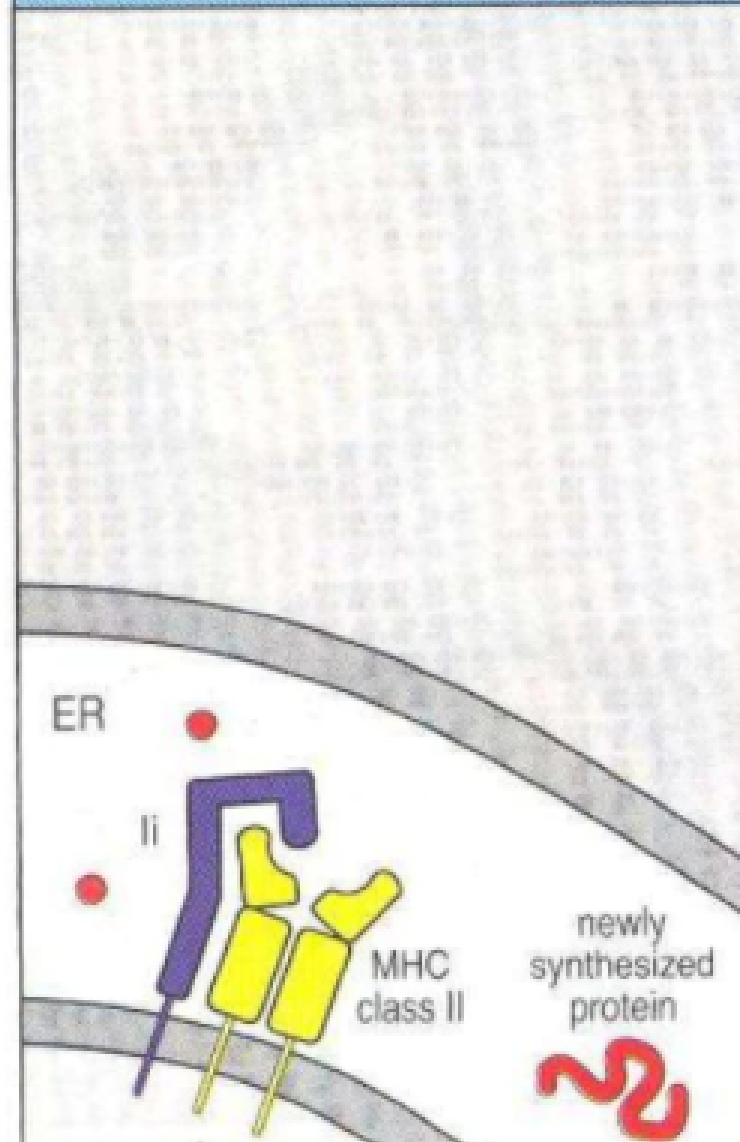


Assembly of class II MHC molecules

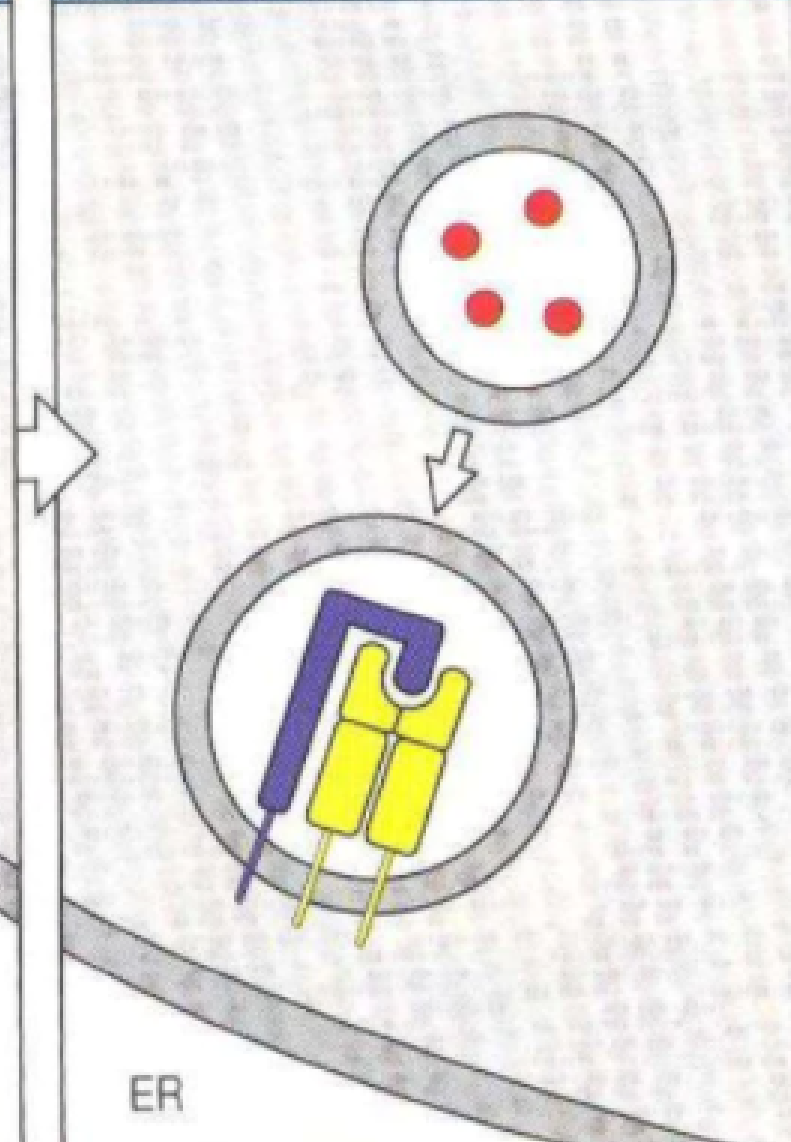
- Within the rough endoplasmic reticulum, a newly synthesized class II MHC molecule binds an **invariant chain**.
- The bound invariant chain prevents premature binding of peptides to the class II molecule and helps to direct the complex to endocytic compartments containing peptides derived from exogenous antigens.
- Digestion of the invariant chain leaves **CLIP**, a small fragment remaining in the binding groove of the class II MHC molecule.
- HLA-DM, a nonclassical MHC class II molecule expressed within endosomal compartments, mediates exchange of antigenic peptides for CLIP.



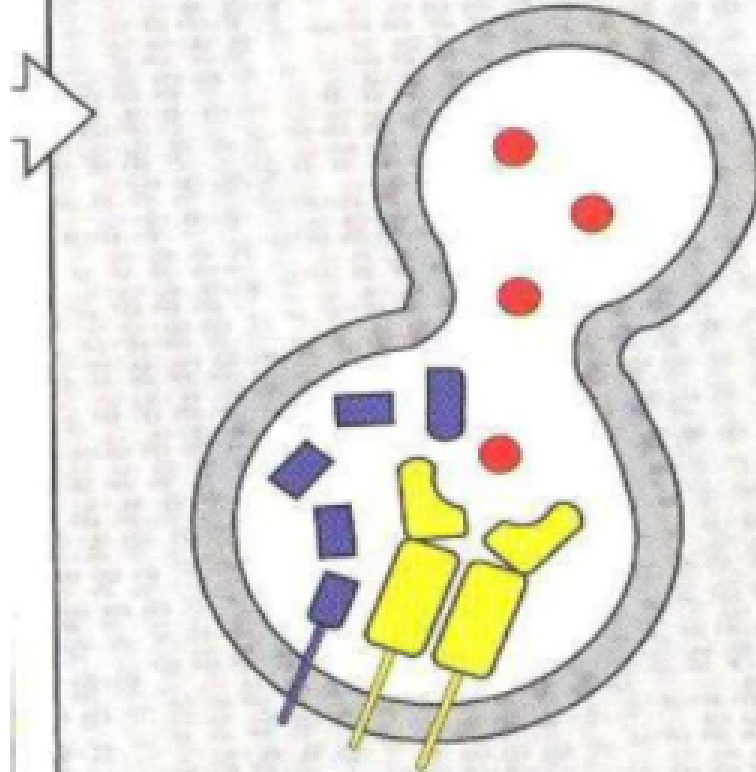
Partially folded MHC class II
is bound by the
invariant chain (Ii) in the
endoplasmic reticulum (ER)



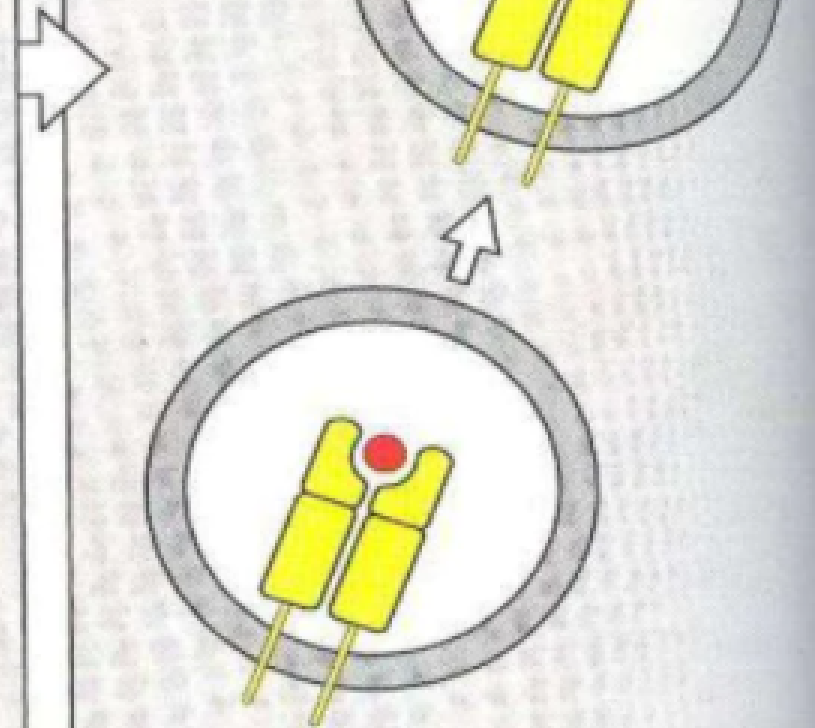
Ii blocks peptide binding
to MHC class II
but facilitates its export
from the endoplasmic reticulum

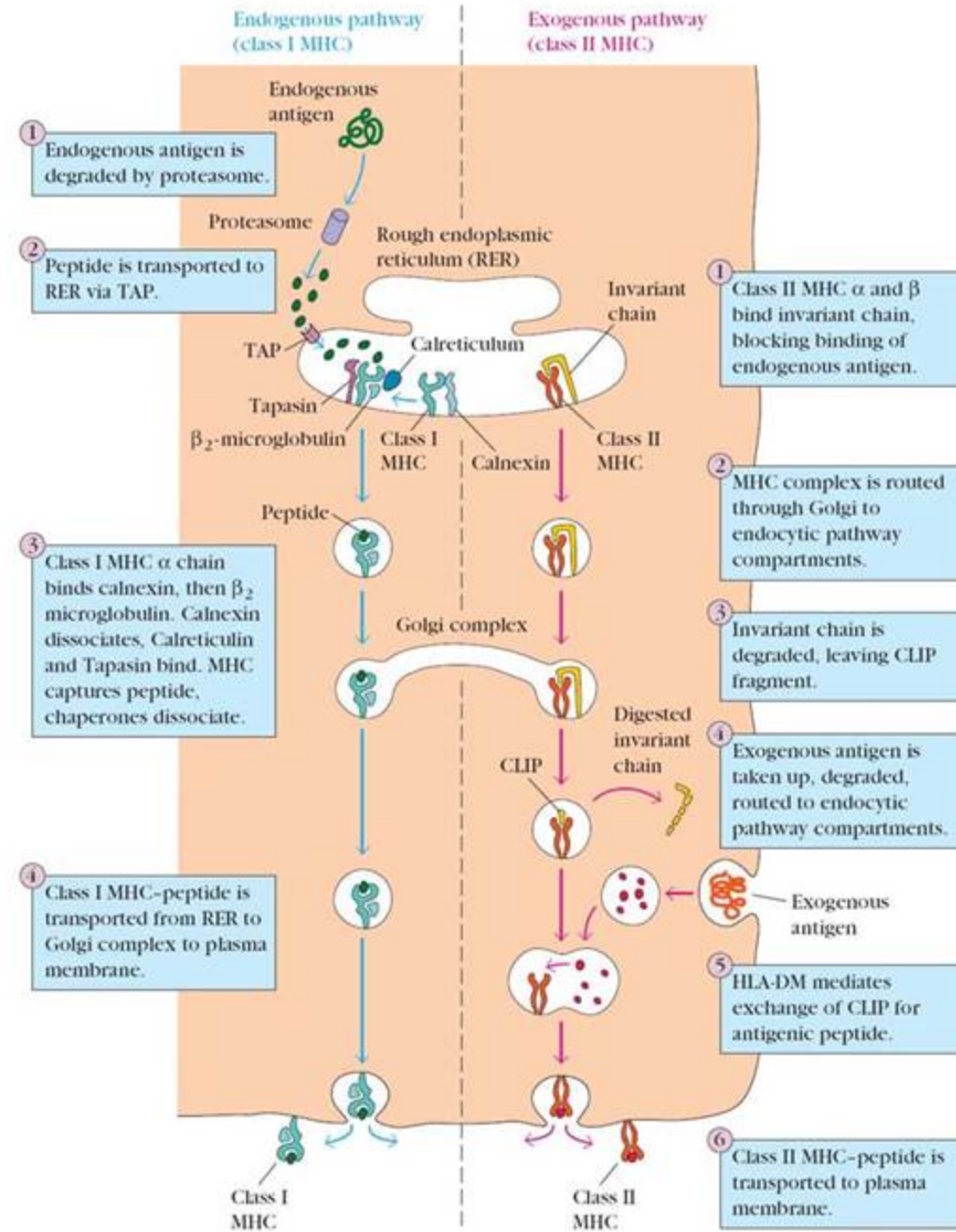


Ii is cleaved in two stages.
Partial cleavage first
may allow MHC class II
to bind incoming antigens

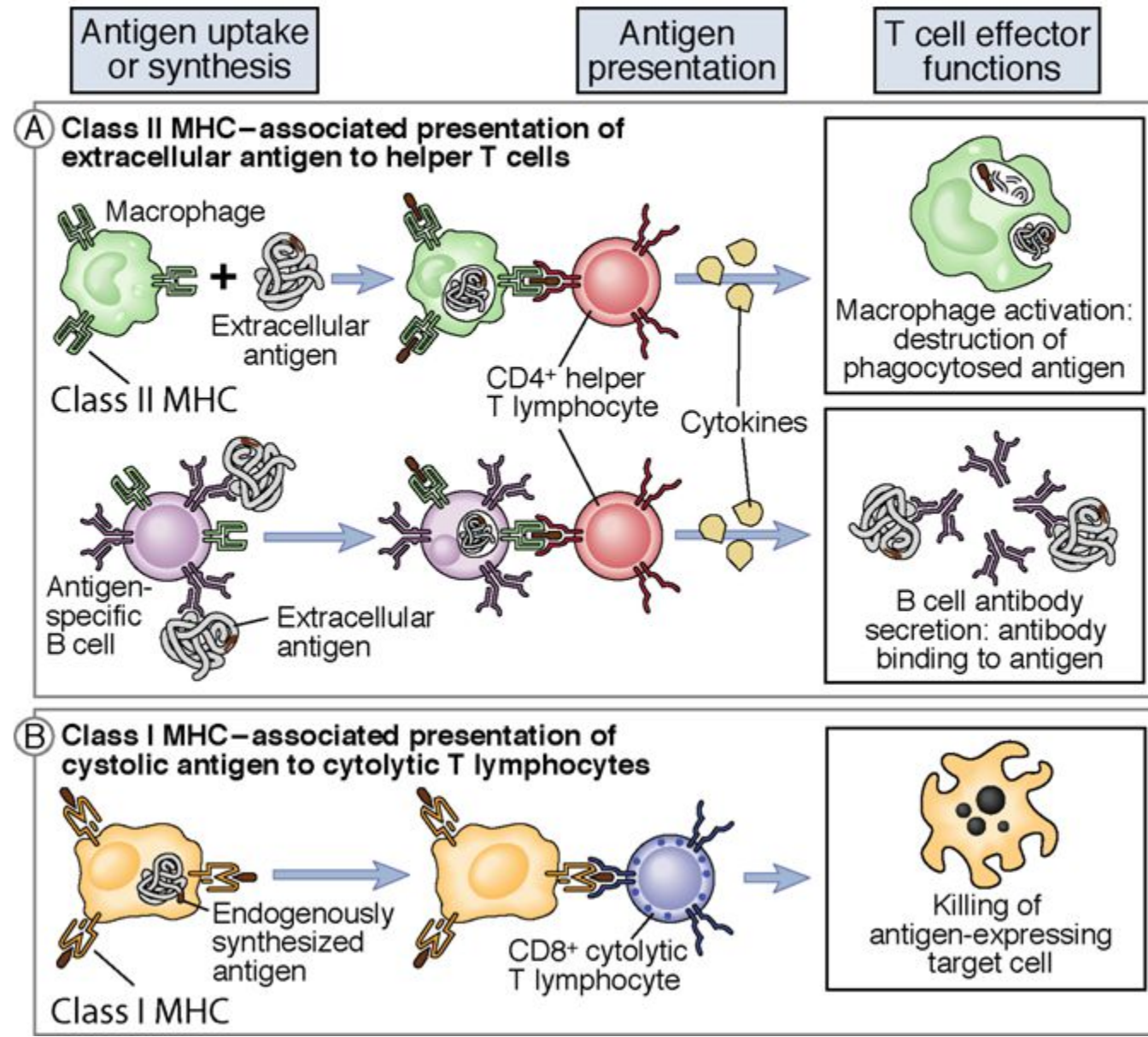


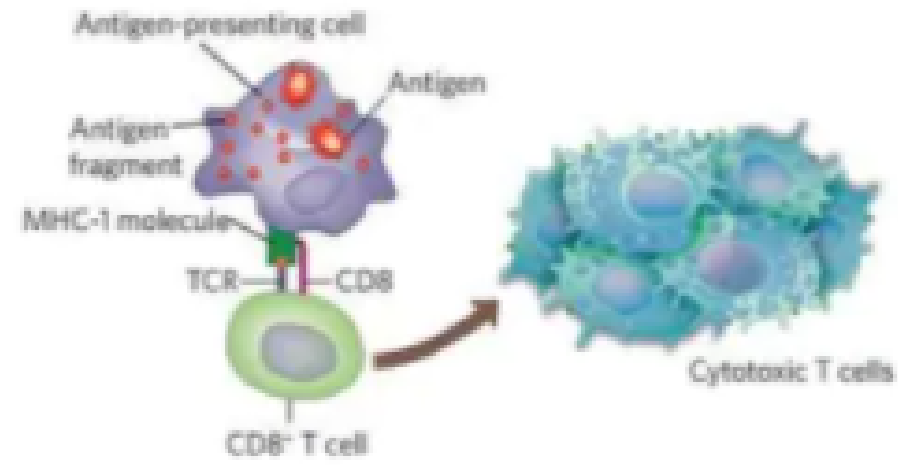
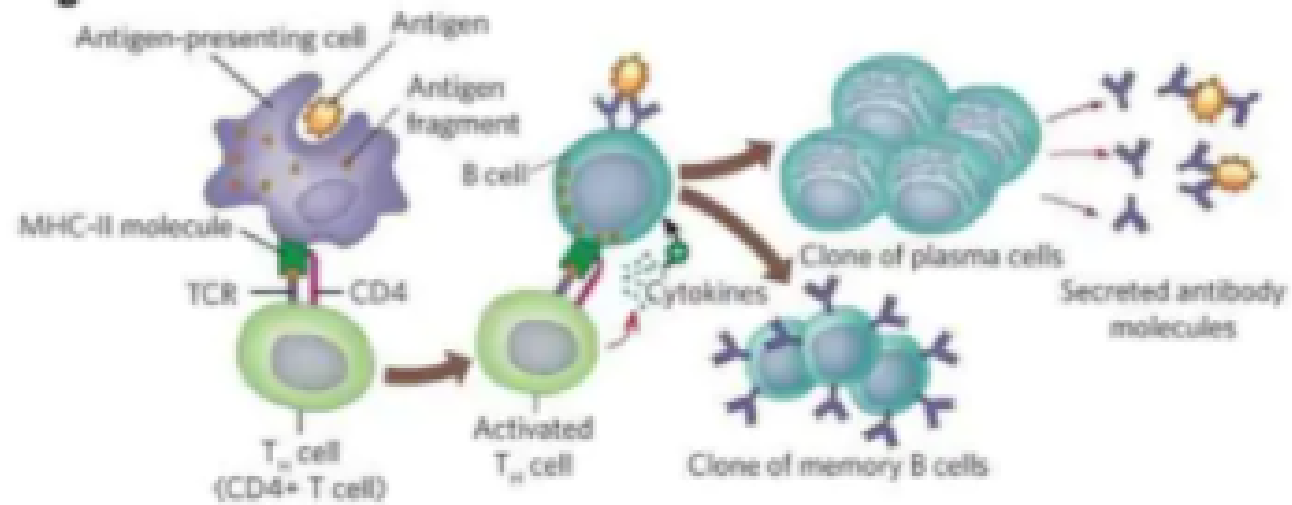
Complete dissociation of Ii
releases MHC class II
to carry antigenic peptide
to the cell surface





How class I- and class II-associated antigen presentation influence the nature of the host T cell response



a**b**



Activation and function of lymphocytes

Malak Alshaikhali

MD, Al-Quds University

MSc Integrated Immunology, University
Of Oxford.

Learning outcomes

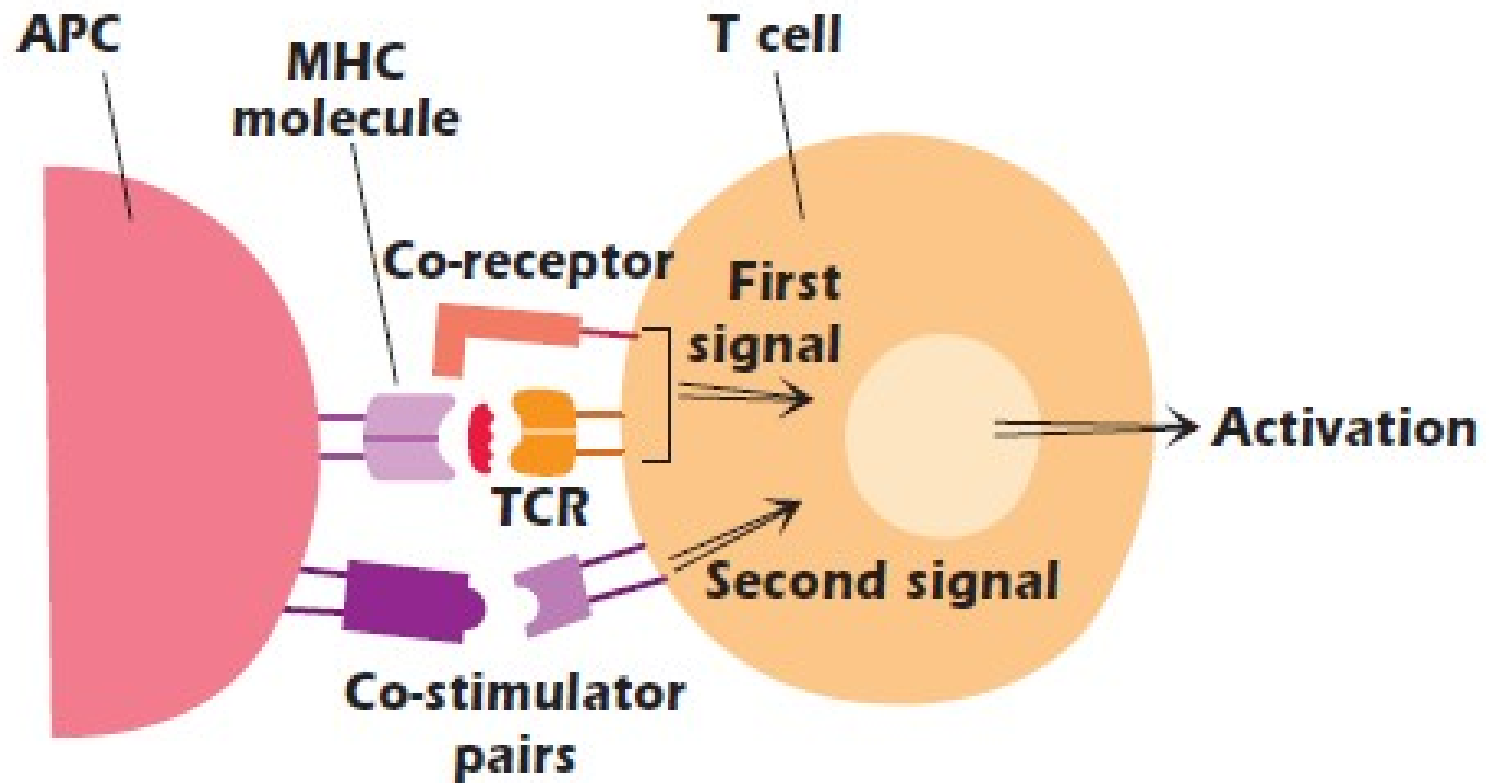
- Understand the activation of T-cells to perform its effector function.
- Understand cytokines produced by subsets of helper T-cells and their role in antibody synthesis.
- Understand the role of cytotoxic killer T-cells.
- Understand the termination of T-cells responses.

The Two signals model for T-cell activation

Naïve T cells require two signals to be activated:

- **First signal** is antigen specific: the interaction between peptide-MHC complex on APC and TCR — the co-receptor signals is also part of the first signal.
- **Second signal:** co-stimulatory molecules.
- Both signals deliver messages to the nucleus which lead to survival, proliferation, and differentiation of the T cell.
- In the absence of the second signal, the first signal alone turns the T cell off rather than on.

Two signals model



Dendritic cells are the main APC that activate Naïve T-cells.

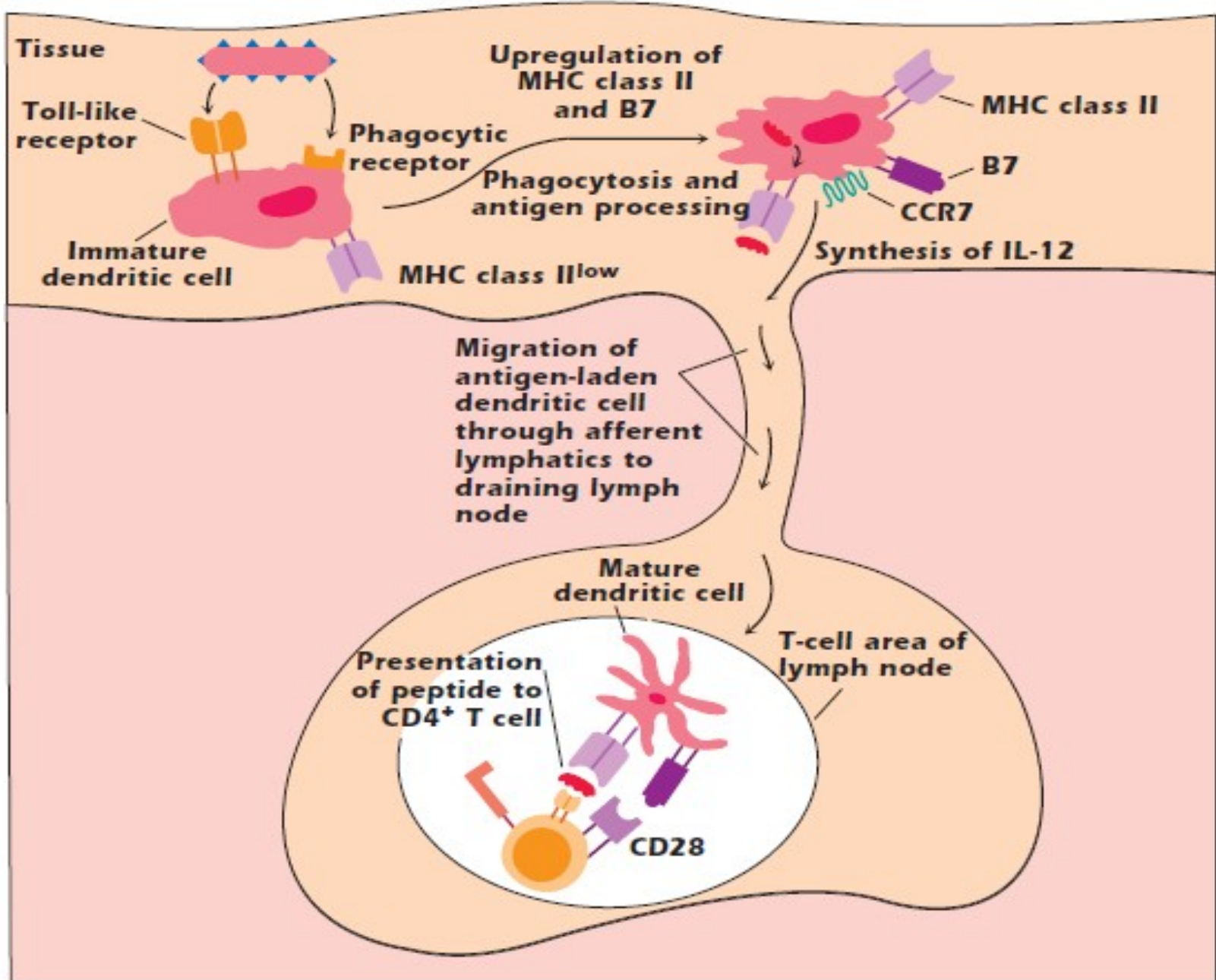
DC are the principal APCs for initiating the primary responses of both CD4+ and CD8+ T cells.

Two main subsets of DC:

1. **Plasmacytoid dendritic cells** synthesize the interferons (IFNs) α and β — play role in innate phase of immune response to pathogens
2. **Myeloid dendritic cells** involved in the induction of T-cell responses.
 - Sites of DC??
 - Migratory vs. lymphoid organs resident, immature vs. mature.

DCs activation

- **Immature DC:** DCs in tissues that has not interacted with antigen.
- **Mature DC:** maturation is triggered by the uptake and processing of a pathogen.
- In the absence of signals induced by pathogens, immature dendritic cells express low levels of co-stimulator and MHC class II molecules— antigens that do *not* induce high levels of co-stimulator function do not activate naïve T cells e.g. small proteins or peptides in vaccines.
- This requirement is essential for prevention of self-responses.
- Adjuvants in vaccines helps DC activation.



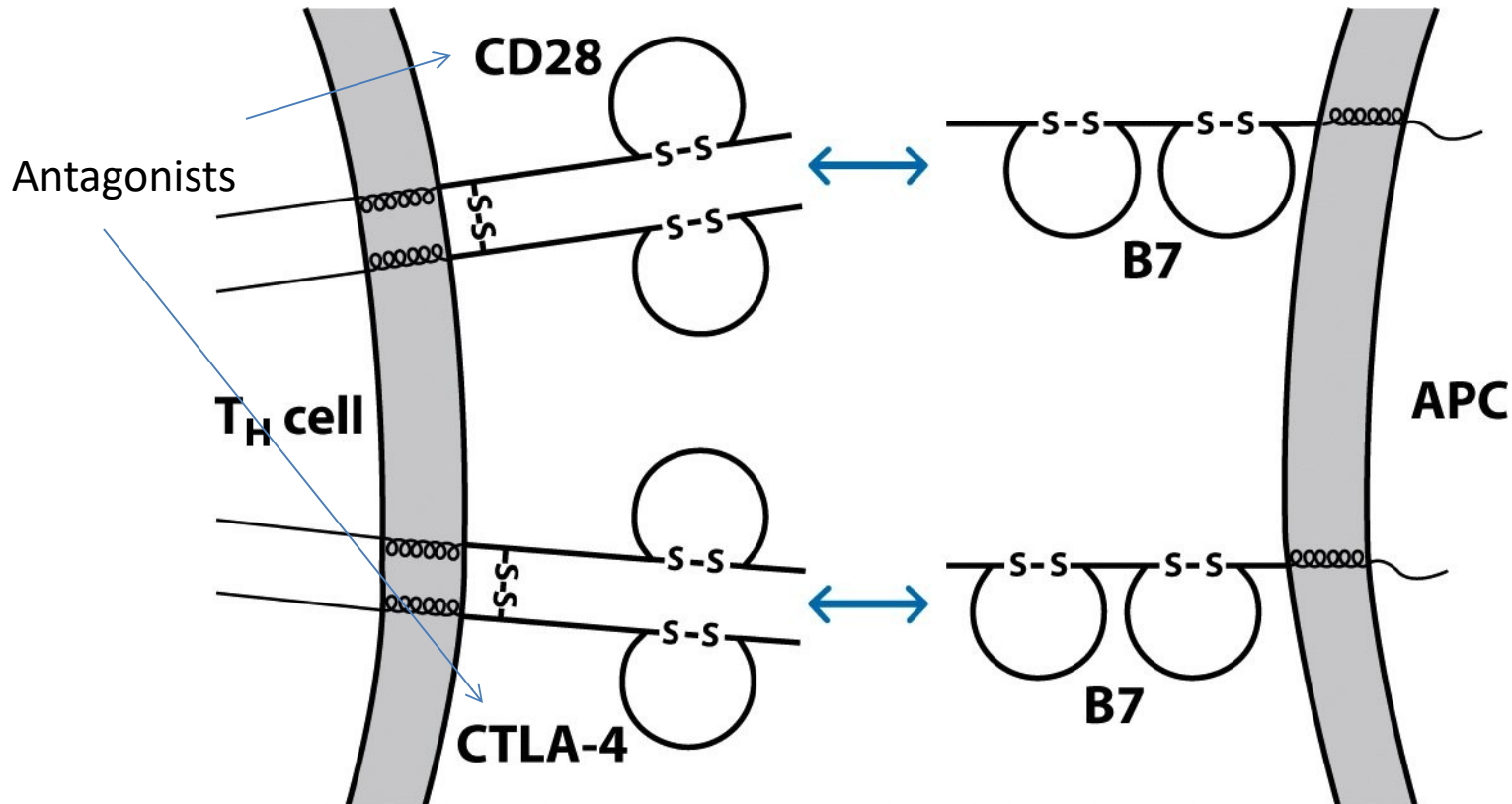
CD4 T cells activation

- The interaction between T-cells and APC forms **immunological synapse**.
- Immunologic Synapse is the area of contact between the CD4+ and APC— contact lasts hours~8hrs
- The synapse incorporates the MHC–peptide and TCR, CD4, and pairs of co-stimulator and adhesion molecules.
- **Co-stimulatory molecules:**
 - The interaction of peptide–MHC with the TCR upregulates T-cell expression of CD40L —it in turn induces increased expression of CD40 on the APC.
 - The B7–CD28 interaction is a critical positive signal in CD4+ T-cell activation.
 - Activation of the T cell also induces expression of **CTLA-4**.

CD28 is expressed by both resting and activated T cells

Costimulatory Signal

CD28 – B7
Activation of
TF for IL-2



CTLA-4 is expressed on activated T cells

Both B7 molecules are expressed on dendritic cells, activated macrophages, and activated B cells

Figure 10-14
Kuby IMMUNOLOGY, Sixth Edition
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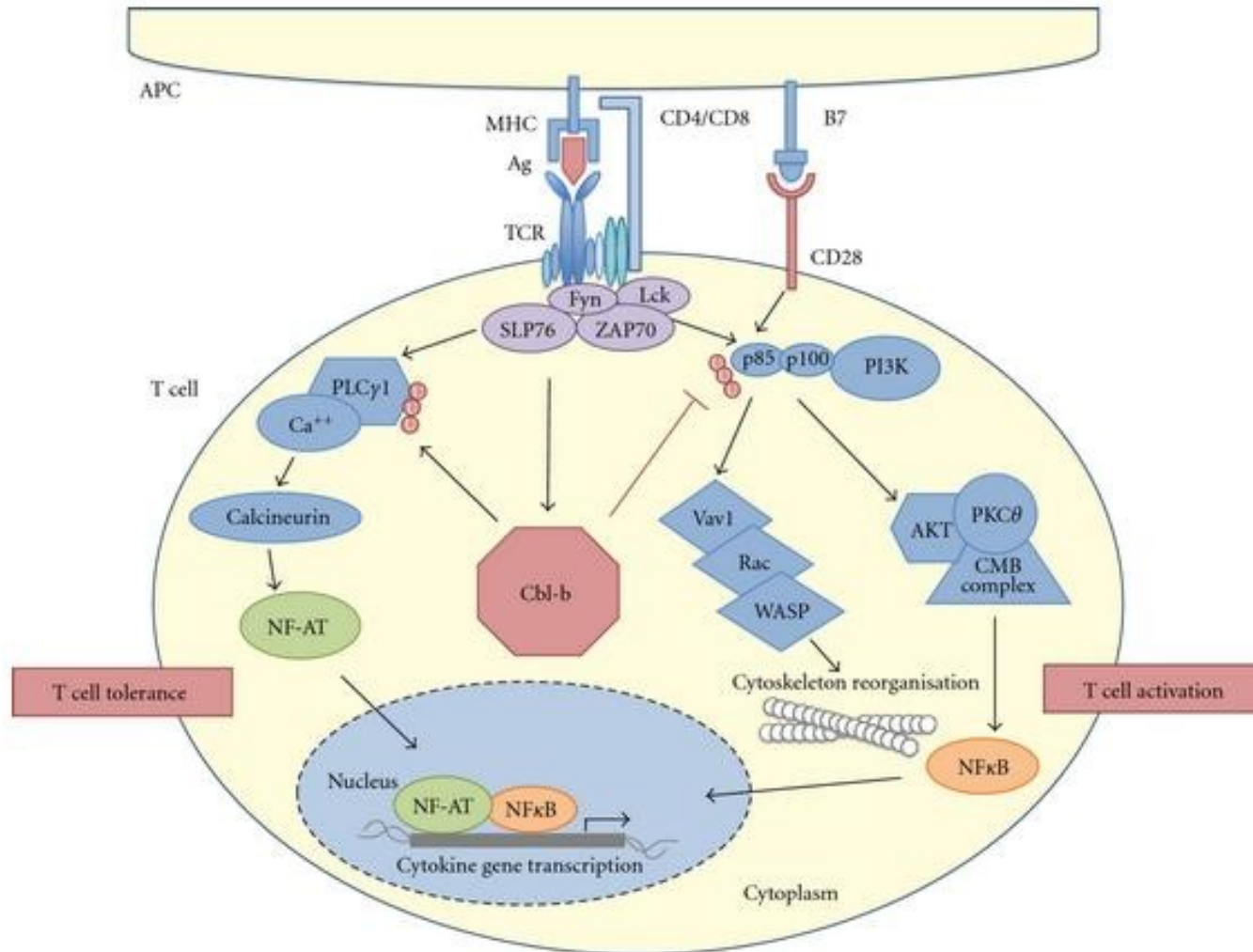
CD4 T cells activation

- At the synapse the first and second signals are transmitted which initiate intracellular events.
- Intracellular events involves activation of Kinases, Phosphorylation of ITAMs, Assembly and activation of signaling complexes at the cell membrane.
- For example: recruitment of **ZAP70** , a tyrosine kinase, to the cell membrane is critical for T-cell activation.
- Individuals who lack ZAP-70 do not respond to antigen and are immunodeficient.

CD4 T cells activation

- A multiprotein complex of signal transduction molecules is assembled in sequence and activated on the cytoplasmic side of the T-cell.
- Ultimately signals lead to the activation of transcription factors that enter the nucleus and alter gene expression.
- These activated transcription factors upregulate expression of **IL-2** and **IL-2R α** .

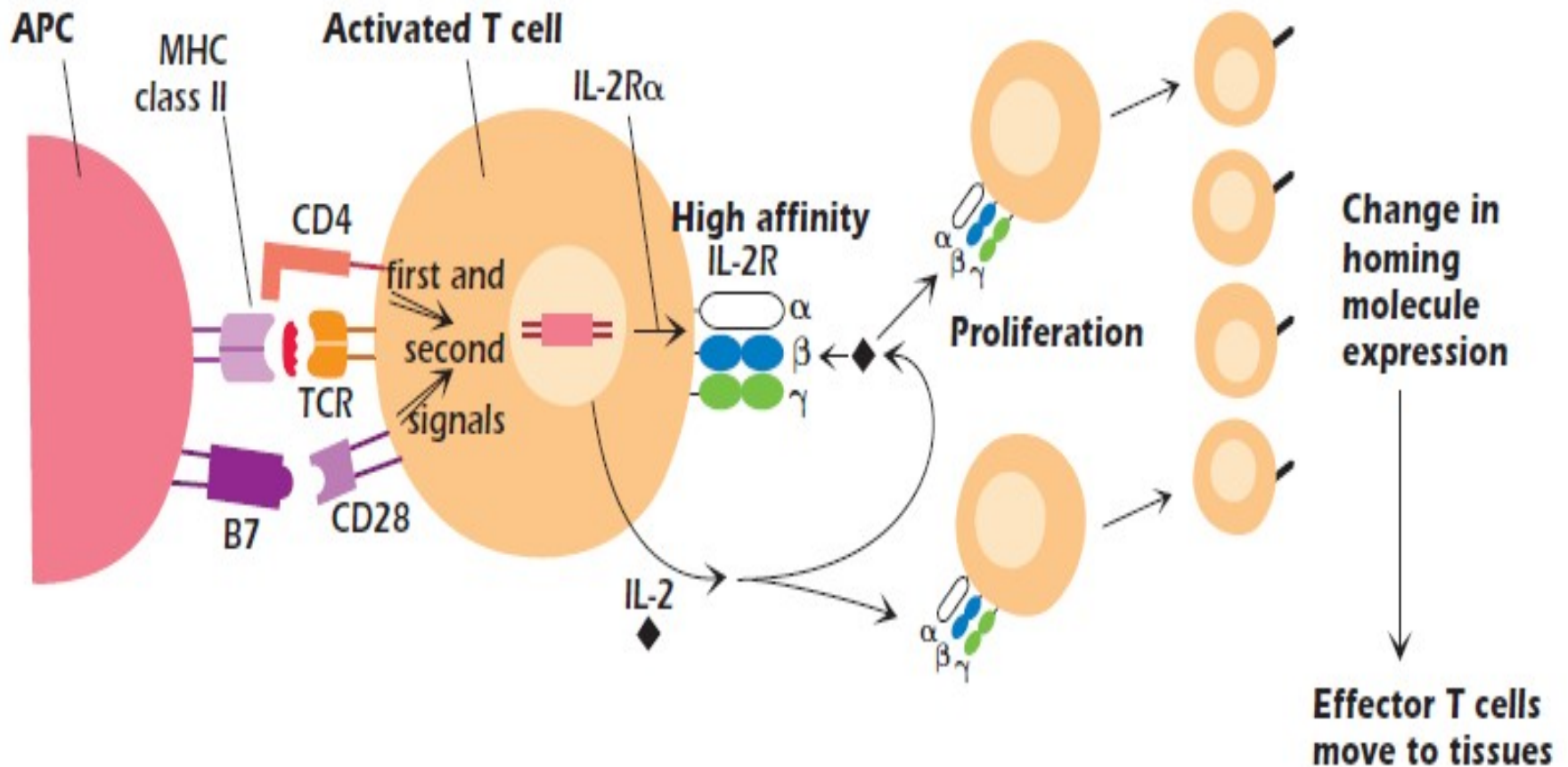
CD4 T cells activation



CD4 T cells activation

- IL-2 is a growth factor for T cells and acts on any cell that expresses the high-affinity IL-2 receptor — only expressed on activated T-cells.
- IL-2 synthesis when there is co-stimulation is 100x over synthesis induced through TCR signaling alone.
- As a result, the cell proliferates and expands the initial clone size and differentiates into an effector cell — migrate out of LN and carry out its specific function.

CD4 T cells activation



CD4 T cells activation

Termination of T cell responses:

- Several pairs of molecules expressed on the T-cell–APC surfaces convey *negative* signals to the activated T cell—turn off signals.
- These negative signals include inactivation of molecules activated during the response, inhibition of the cell cycle, and the induction of T-cell exhaustion.
- Example of negative signaling molecules: CTLA-4 and PD-1.

CD4 T cells activation

- **CTLA-4:** competes with CD28 for binding with B7 molecules on APC.
 - The expression of CTLA-4 turns off the production of IL-2 and thus ends T-cell proliferation, limiting the extent of the immune response.
- ***Programmed cell death protein* (PD)-1**
 - Member of the CD28/CTLA-4 family.
 - Expressed on activated CD4+ and CD8+ T cells— associated with T-cell exhaustion.
 - It conveys a negative signal to the activated T cell, by inhibiting ZAP-70 function— it binds PD-L1&2 expressed on normal cells, DC and tumor cells.

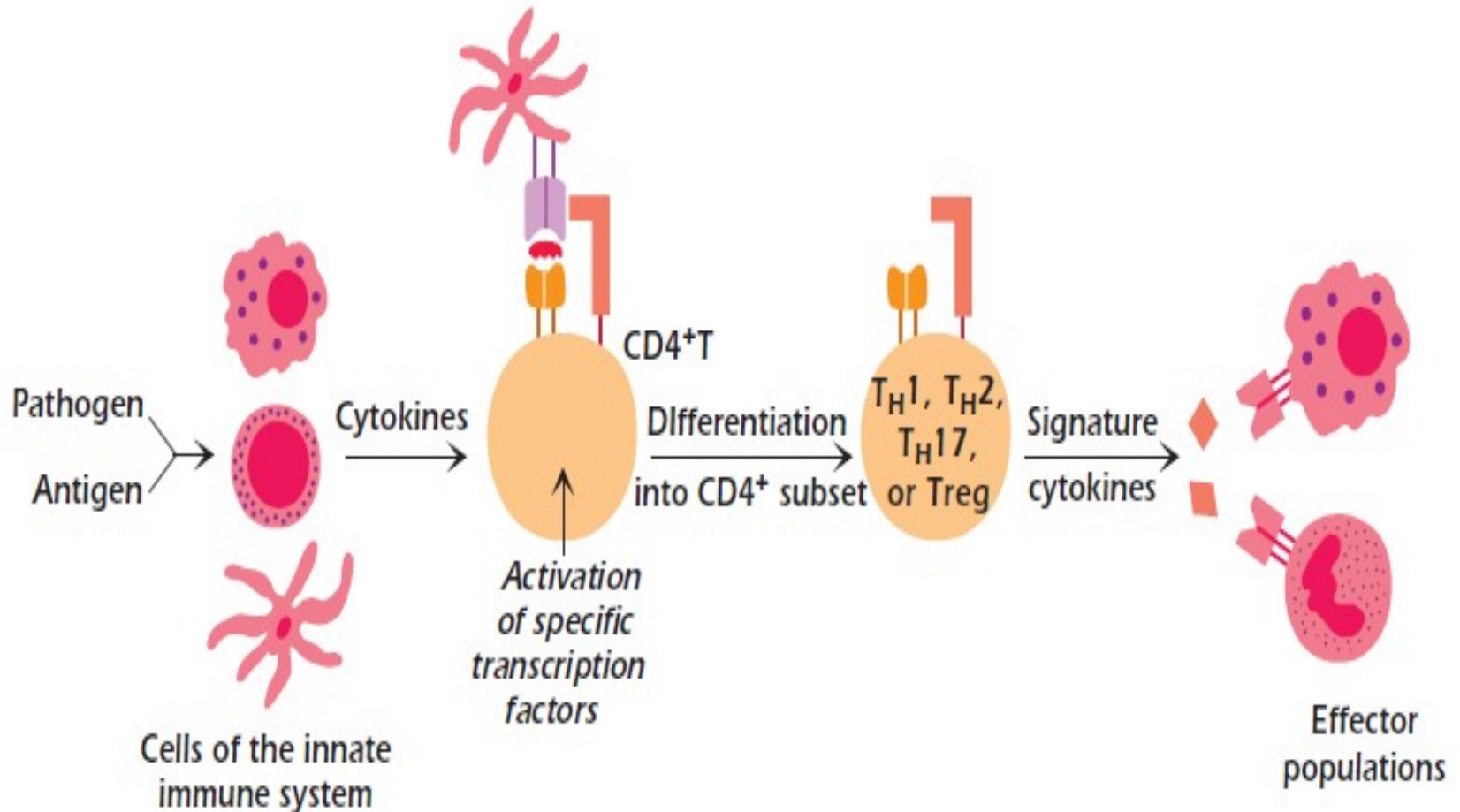
CD4+ T cells subsets and functions

- Cytokines present during the activation of naïve CD4+ T cells drive the differentiation of the CD4+ T cell into subsets: ***TH1***, ***TH2***, ***TH17***, or ***Treg*** cell —referred to as the third signal.
- These cytokines are produced early in the response by innate immune cells.
- They activate “lineage-determining” transcription factors in the CD4+ T cell — this determines which subset is produced.
- Each subset of CD4+ T cells synthesizes a unique set of ***signature cytokines*** — e.g “TH1-type” or “TH2-type” response.

Th0 cells

- **Th0** cells refer to the naïve T cells or the first T cells produced after antigen stimulation.
- Depending on the stimulating antigen and the cytokine present, the Th0 could then further differentiate into either a Th1 or Th2 cell.
- Each subset of CD4+ T cells interact with a unique set of effector cells, which deal with a specific type or types of pathogen.

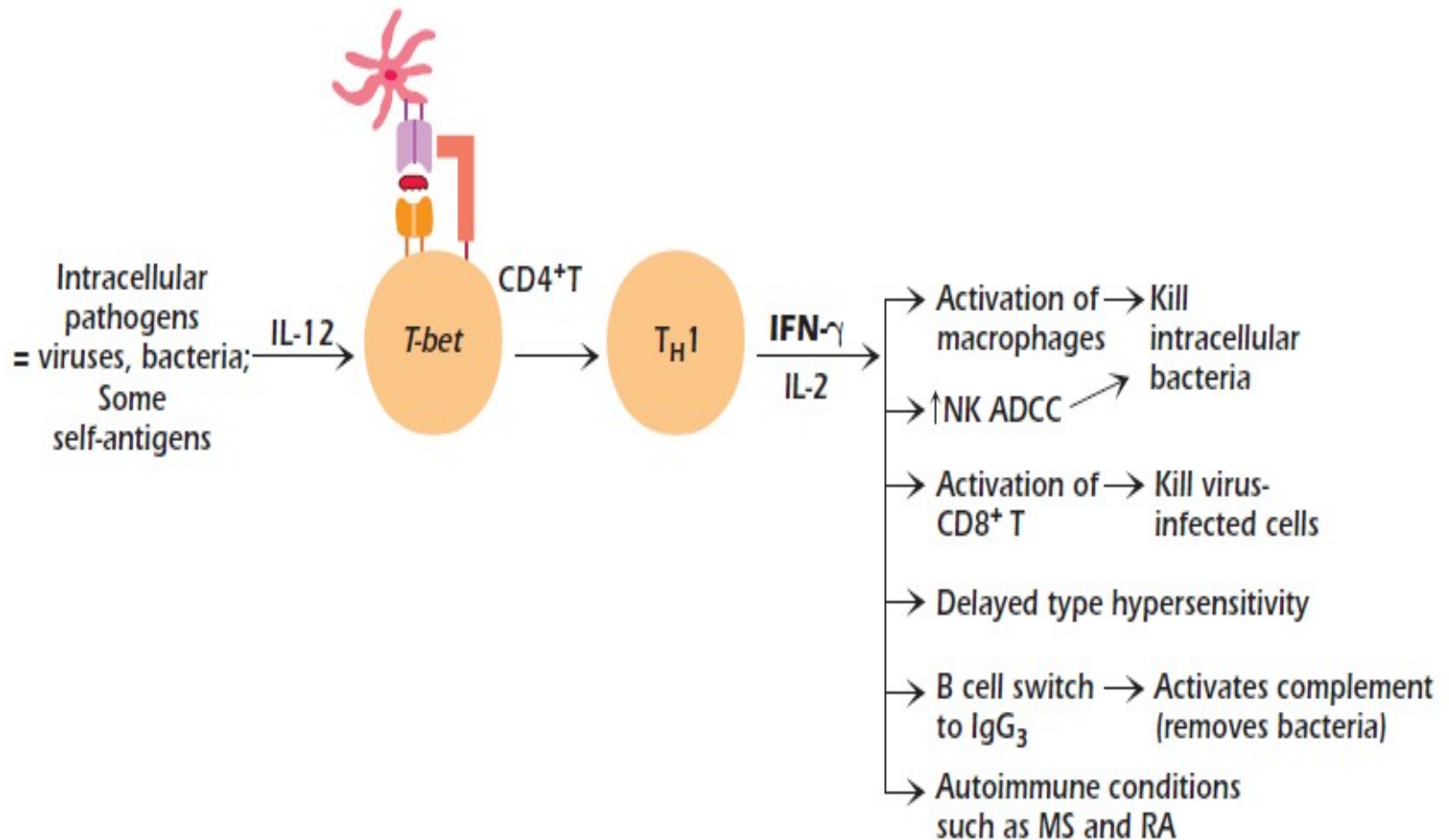
T_H0 cells



Th1 cells

- Develop in the presence of IL-12.
- IL-12 source: innate cells eg DC responding to intracellular pathogens.
- IL-12 activates the lineage-specific transcription factor **T-bet**- it induce synthesis of **IFN- γ** and **IL-2**
- **Function:**
 - They activate macrophages, CD8+ T cells, and NK cells— kills intracellular pathogens.
 - Involved in type IV hypersensitivity.
 - IFN- γ influences B-cell class switching: IgG3— enhances phagocytosis.
 - Involved in autoimmune diseases e.g. RA and MS.

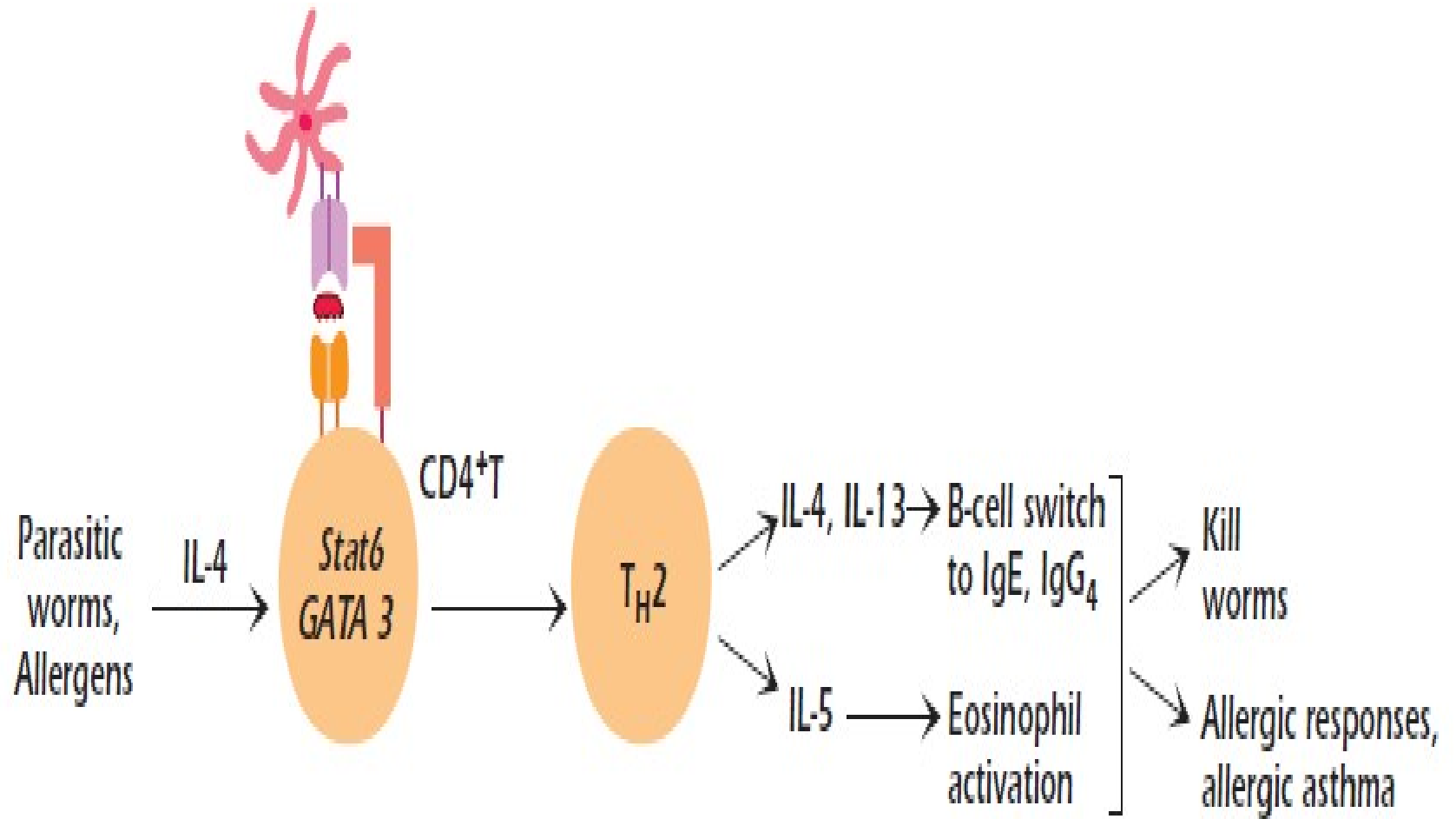
T_H1 cells



Th2 cells

- Develop in the presence of IL-4, early in the response to parasitic worms such as helminths and to allergens.
- IL-4 source? mast cells, activated CD4+ T cells, and NKT.
- IL-4 activates **GATA3**, the lineage determining transcription factor- it induce synthesis of **IL-4, IL-5, IL-13**.
- **Function:**
 - Immune responses to parasitic worms such as helminths.
 - IL-4 and IL-13 : B-cell class switch to IgE and IgG4.
 - IL-5 activates eosinophils.
 - Responses to allergens: type I hypersensitivity.
 - Involve in allergic asthma.

Th2 cells



Th17 cells

- Develop in the presence of TGF- β , IL-21, and IL-23.
- Source: activated CD4+T cells (IL-21), innate cells.
- Activates **ROR γ t**, the lineage-determining transcription factor- induce synthesis of **IL-17, IL-21, IL-22**.
- **Function:**
 - Provide protection at mucosal surfaces.
 - involved in responses to extracellular bacteria and fungi.
 - Role in autoimmune conditions that involve chronic inflammation e.g RA, MS, IBD, and psoriasis.
 - Express **RANKL** which activate osteoclast and stimulate bone resorption— role in bone destruction in RA.

Treg cells

- **Induced** (or **iTreg**)—develop in the periphery from naïve T cells in the presence of TGF- β .
- Express the lineage determining transcription factor **Foxp3**.
- Treg also express CD25 (the α chain of the IL-2 receptor) and CTLA-4.
- They produce cytokines TGF- β and IL-10.
- **Function:**
 - inhibit the differentiation and function of the other subsets of CD4⁺ T cells and other cell types e.g. DC and B-cells.
 - suppress immune responses directed at both self-molecules and foreign antigens — play a role in tolerance and regulation.

Cross-Inhibition of CD4+ T-Cell Subsets

- Cytokines produced by one subset, inhibits the development of other subsets.
- This results in **polarization** or skewing of the immune response towards the production of only one subset of CD4+ T cells— depending on the cytokines produced early in the response by innate cells.

Subset	Cytokine	Inhibits
T _H 1	IFN- γ	T _H 2 and T _H 17
T _H 2	IL-4	T _H 1 and T _H 17
Treg	TGF- β	T _H 1, T _H 2, and T _H 17

Polarised CD4 T cell responses

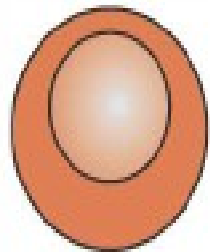
- Th17 cells
 - Typically against extracellular bacteria & fungi
 - Recruit neutrophils to early sites of infection and activate them.
- Th1 cells
 - Typically against intracellular bacteria & protozoa
 - Recruit and activate macrophages.
 - Help B cells to secrete specific IgG subclasses.
- Th2 cells
 - Typically against parasitic helminths
 - Recruit eosinophils & basophils to 'barriers'.
 - Help B cells to secrete e.g. IgE.

Helper T cells subsets

Signal 3 delivered by antigen-presenting cell

TGF- β

FoxP3

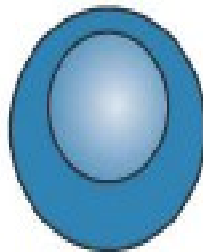


TGF- β , IL-10

T_{reg} cells

IL-6

Bcl6

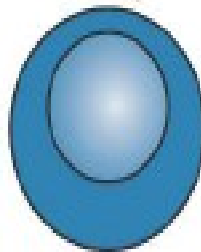


IL-21, ICOS

T_{FH} cells

TGF- β
IL-6

ROR γ T

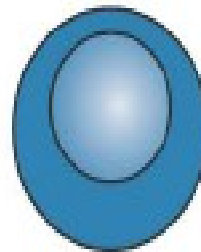


IL-6, IL-17

T_H17 cells

IL-12
IFN- γ

T-bet

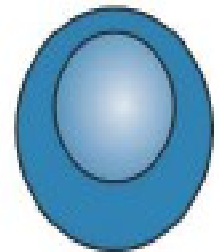


IL-2, IFN- γ

T_H1 cells

IL-4

GATA3



IL-4, IL-5

T_H2 cells

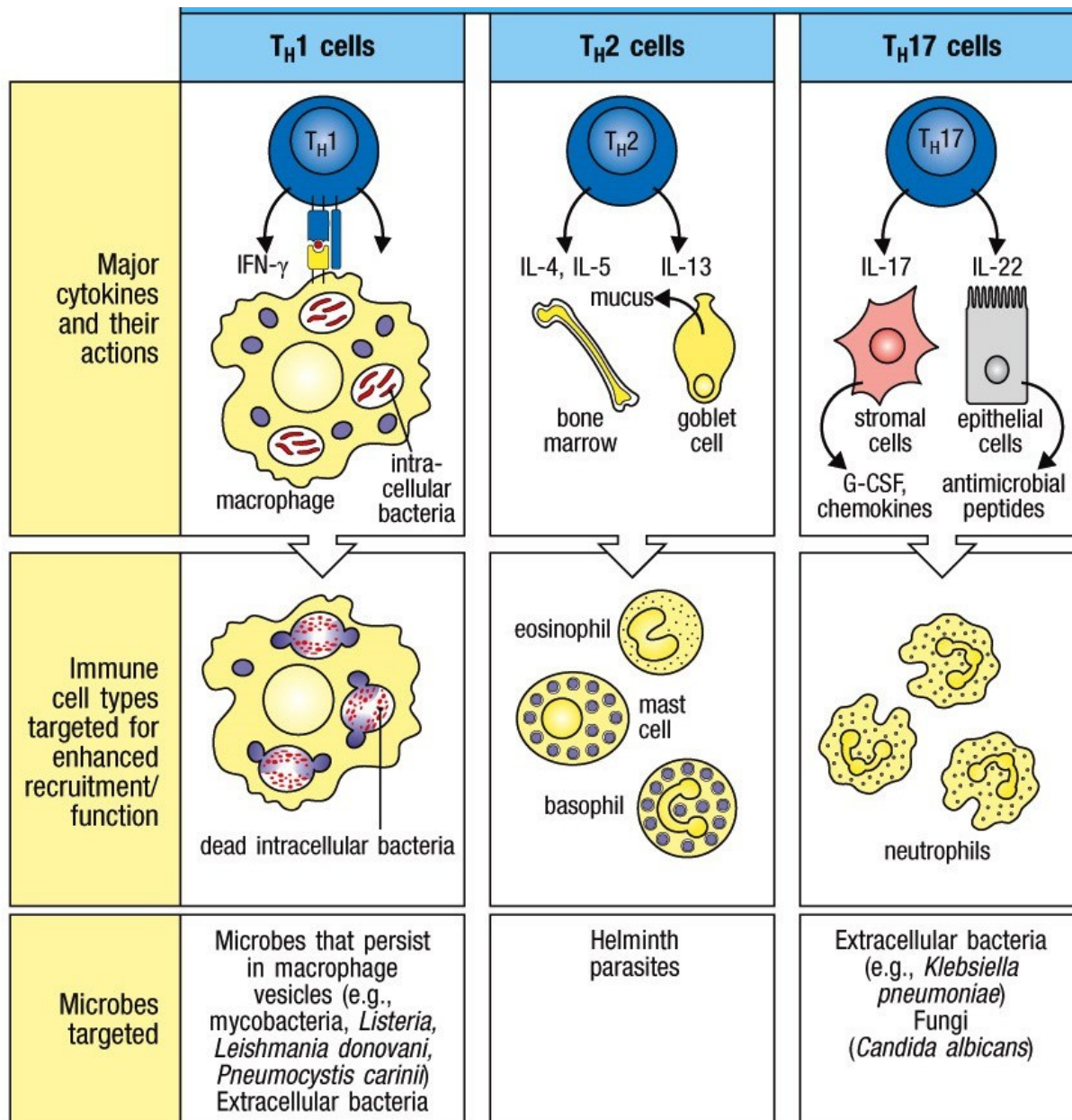
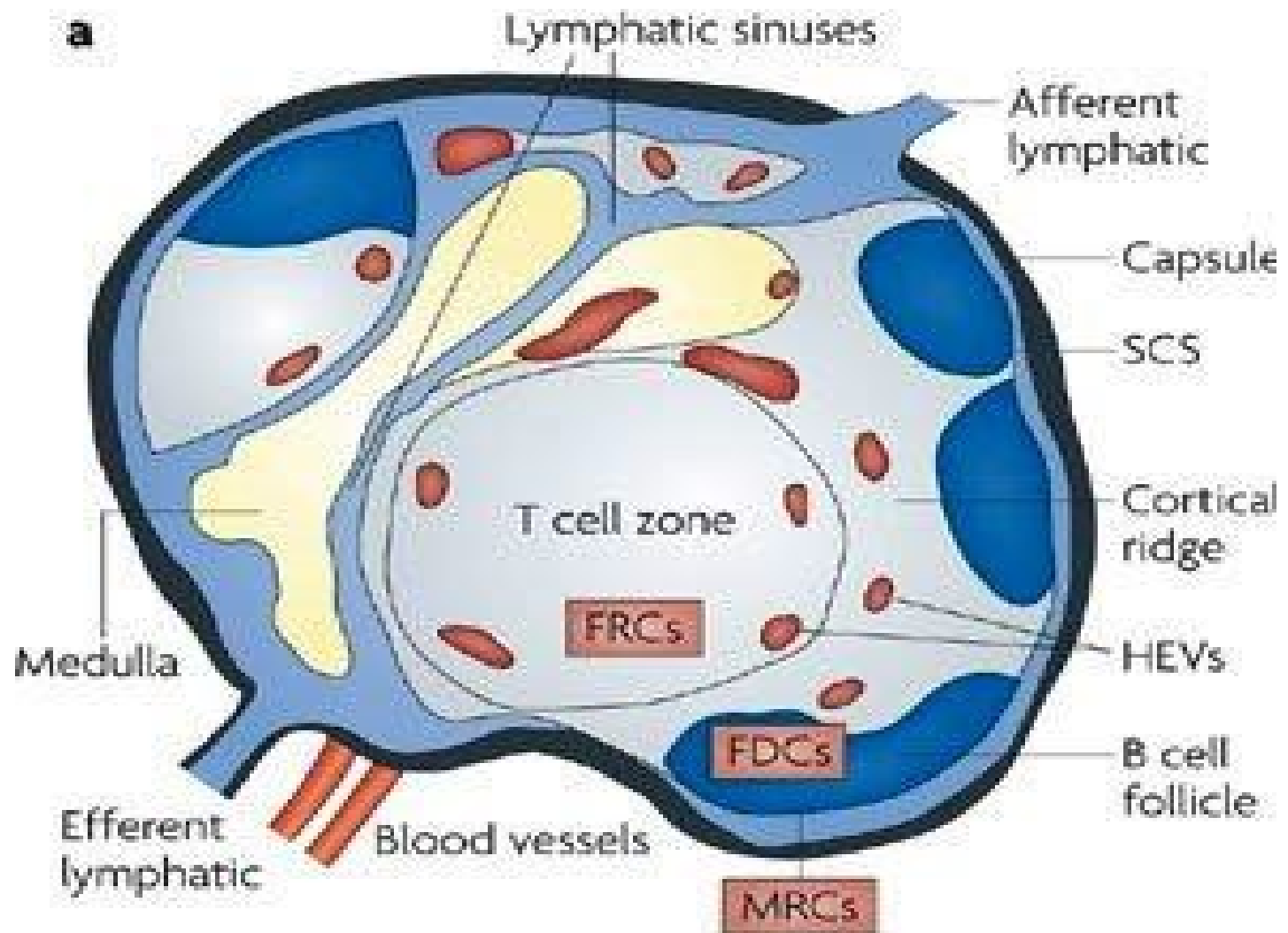


Figure 9.30 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

B cell activation in response to TD antigens.

- B-cell activation, differentiation and synthesis of antibodies occur in the germinal center in the response to thymus-dependent (TD) antigens.
- TFH cells are responsible for this interaction with B cells – the interaction of antigen-activated CD4+ T and B cells induces the expression of the transcription factor Bcl-6, the transcription factor that characterizes TFH cells.

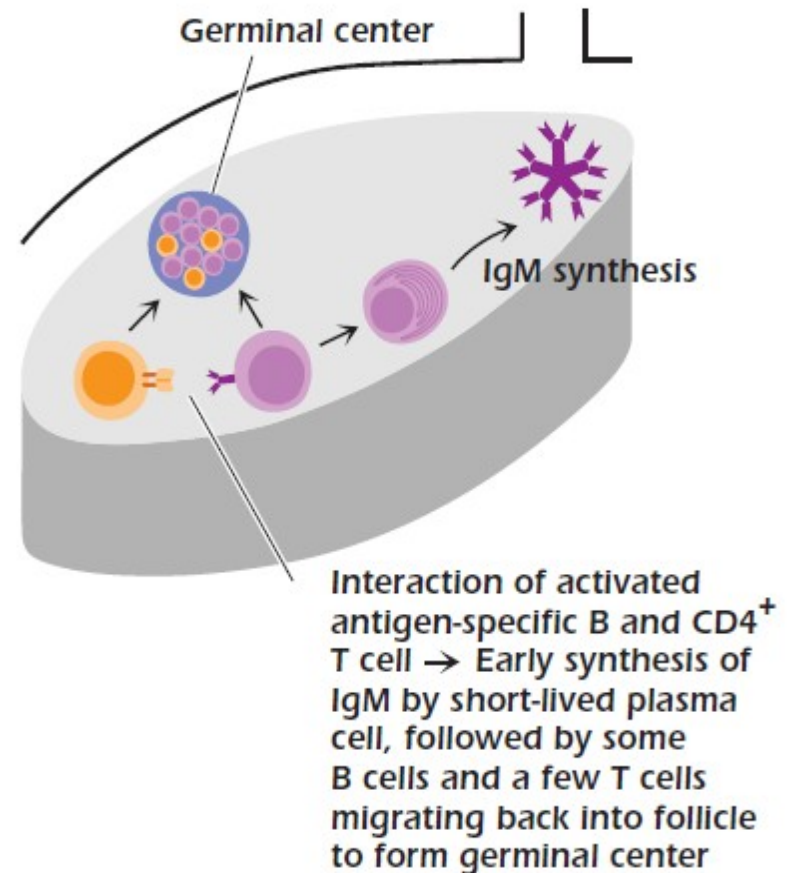
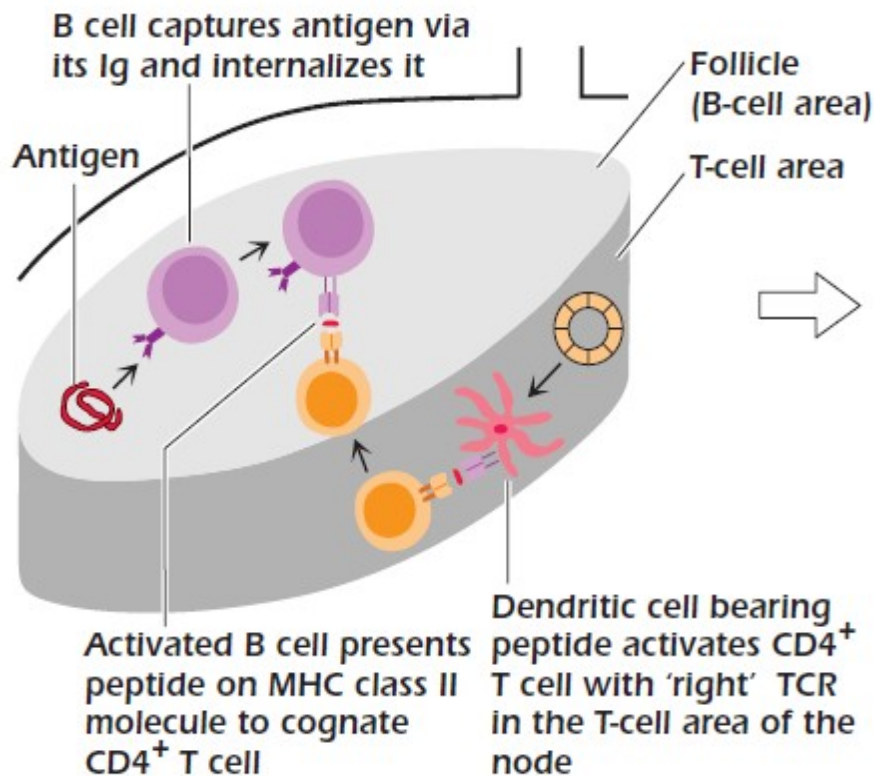


TFH Cells

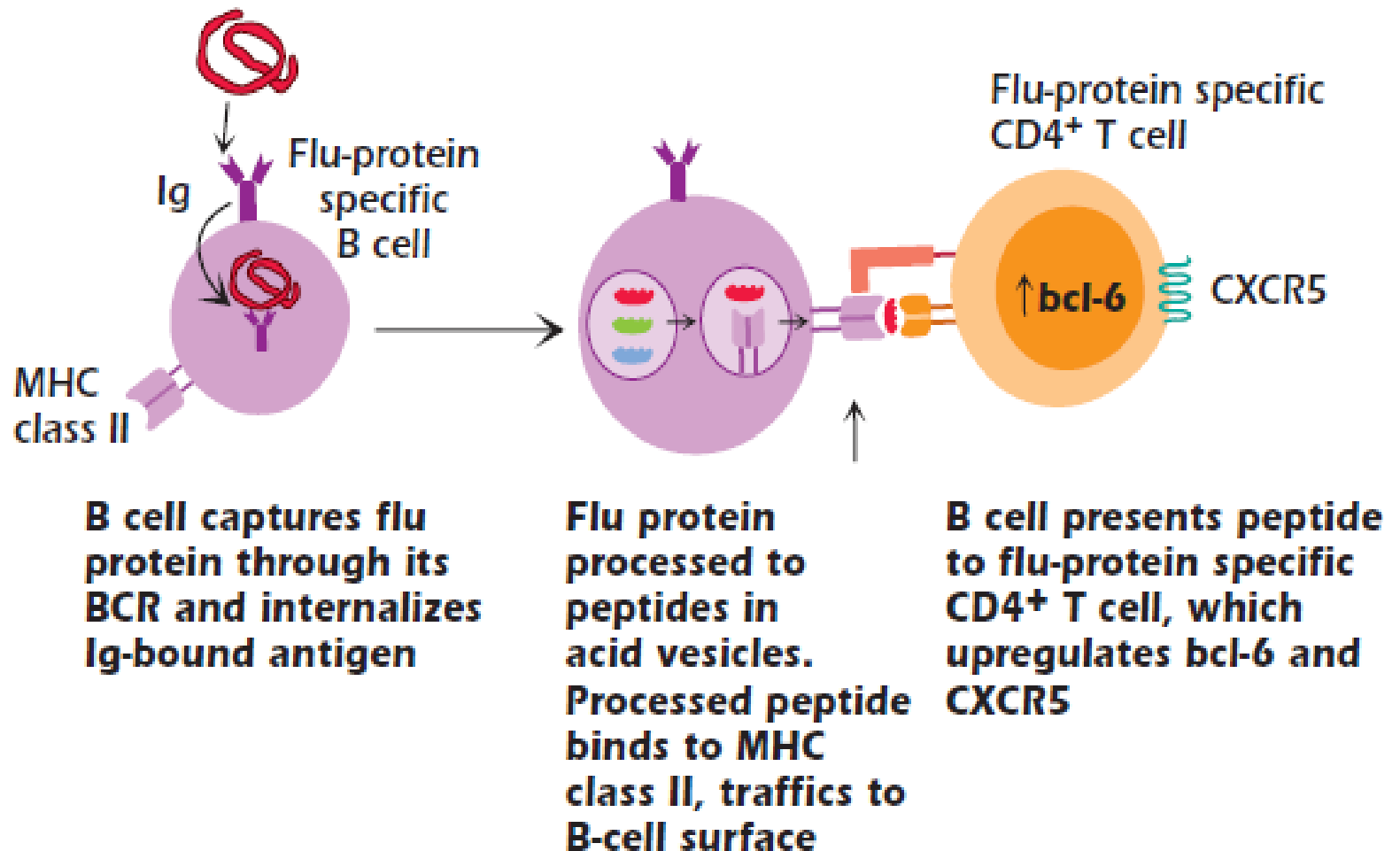
- The CD4+ T cell and B cell specific for the same antigen interact in TD responses —it occur predominantly in the follicles of SLO.
- TFH cells express the transcription factor **Bcl-6**, the chemokine receptor **CXCR5**, and secrete **IL-21**.
- CXCR5 is a marker of the TFH that cooperates with B cells in the GC— its expression is induced by Bcl-6 activation.
- It guides the CD4+ T cell—and **its cognate B cell**— to move into the B cell follicle and the GC develops.

TD B cells activation

- Once cognate B-and T-cells are activated by the specific antigen, they interact with each other at the boundary of the follicle and T-cell area of the node.
- Here, B-cells act as APC for T-cells.
- The interaction between CD4+ T cell and the B cell lasts several hours~ 48hrs.
- The result: B cell differentiate into Plasmablasts that synthesizes IgM, whereas others move back into the follicle to form a germinal center.



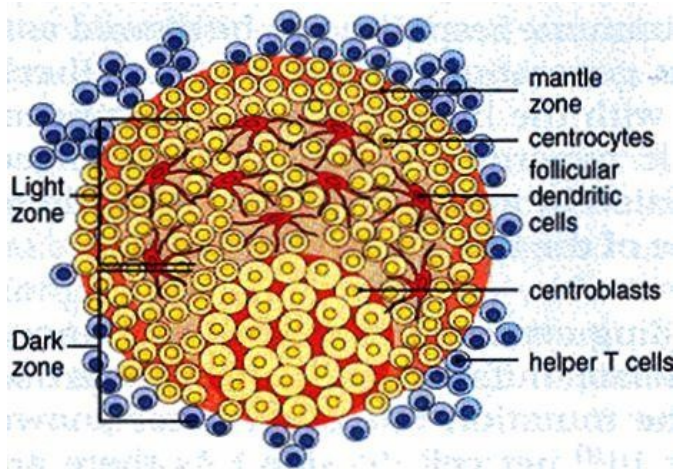
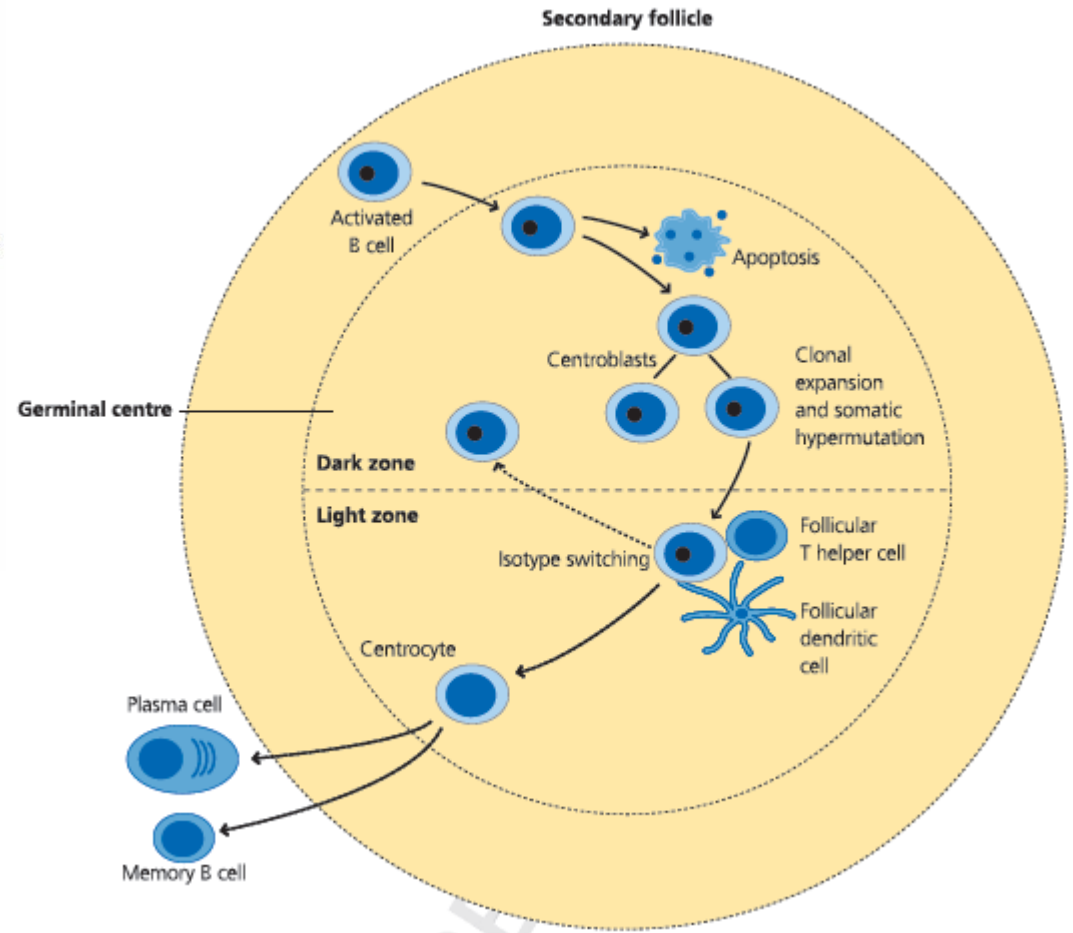
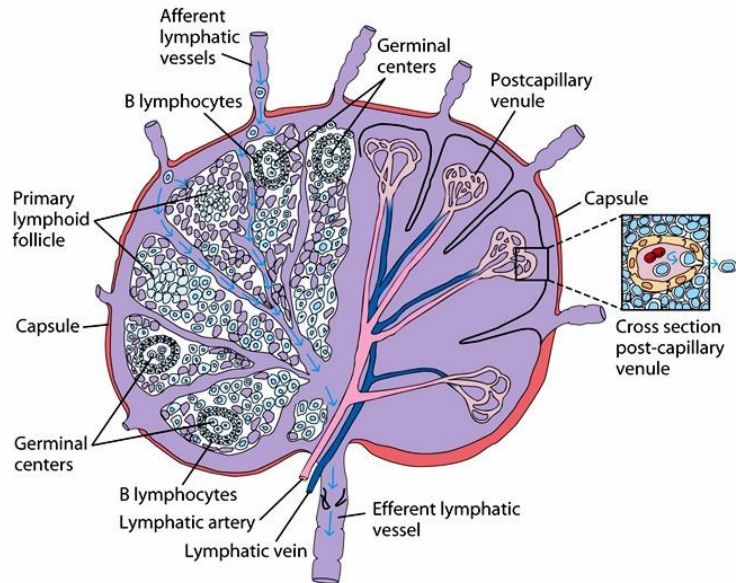
T_{FH} Cells



Germinal centre formation

- After 48hrs of contact, activated B-cells migrate back to the B-cell areas (primary follicles) to form **germinal centre (GC)**.
- GC reactions are important for response to TD antigens.
- Germinal centers are sites of sustained B-cell proliferation and differentiation.
- Follicles in which germinal centers have formed are known as **secondary follicles**.
- **Events that take place at the GC include:**
 - Somatic hypermutation
 - Affinity maturation
 - Class switching.

Germinal centres



GC events

- The interaction between T_{FH} cells and B cells in the GC is similar to the interactions of antigen-bearing dendritic cells and CD4⁺ T cells in the response of naïve T cells.
- Immunological synapse forms: two signals delivered to T_{FH} cells.
- Interaction between pairs of co-stimulators is essential for antibody synthesis and class switching: interactions between CD40 and CD40L, B7 and CD28, and ICOS and ICOS ligand.

GC events

- Interactions between the co-stimulator pairs upregulate TFH cytokine synthesis.
- As a result of the co-stimulator pair and cytokine-cytokine receptor interactions, the B cell undergoes somatic hypermutation and class switching— this give rise to memory B cells and plasma cells.

Somatic hypermutation

- Antigen-activated B-cells proliferate in the GC.
- Ig-Variable region gene mutations occur at the GC dark zone.
- This results in production of B cells that synthesize and express Ig molecules with altered affinity for the activating antigen— higher or lower affinity.
- This process is mediated by the enzyme **activation-induced cytidine deaminase (AID)** — it changes nucleotides in the DNA of Ig variable-region genes

Affinity maturation

- After somatic hypermutation, B cells enter the *light zone* of the GC where they interact with two cells:

1. Follicular DC:

- Not related to the APCs DCs.
- They have long process that bind antigens in Ag-Ab complexes.
- They retain antigens on their surface for long period and present it to B-cells in the GC.

2. T follicular helper cells (Tfh)

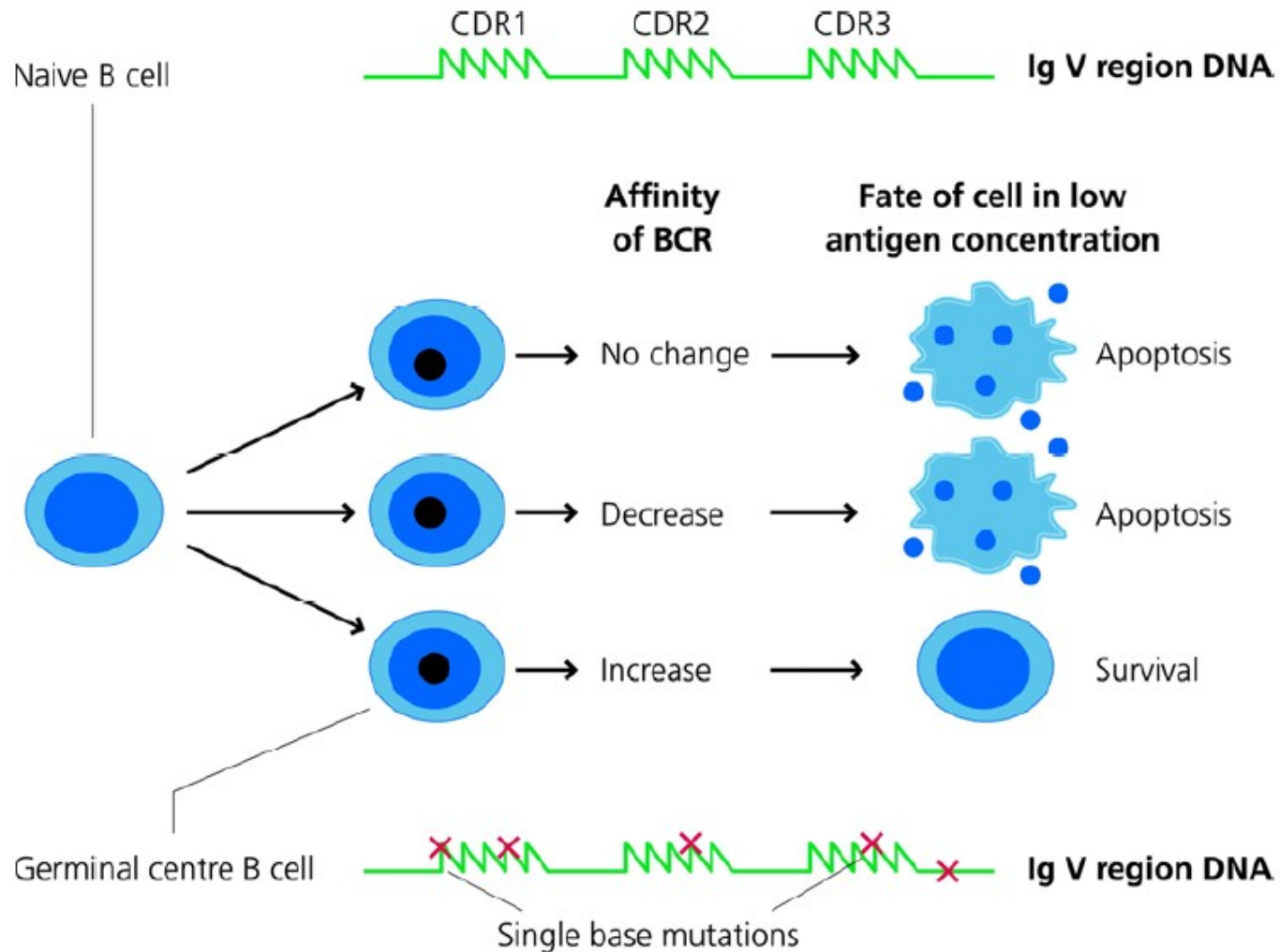
Affinity maturation cont.

- B cells in the GC compete for the antigen presented by follicular DC:
 - ❑ B-cells with the highest affinity Ig to the Ag — clonally selected and expand.
 - ❑ B-cells with low affinity Ig— not selected and die by apoptosis.

Affinity maturation cont.

- The B cells that emerge synthesize antibodies that overall have higher affinity for the activating antigen than the original population of B cells.
- **Tingible body macrophages:** remove the dead cells by phagocytosis — characteristic of reactive follicle.

Somatic hypermutation



B cell selection in germinal centre

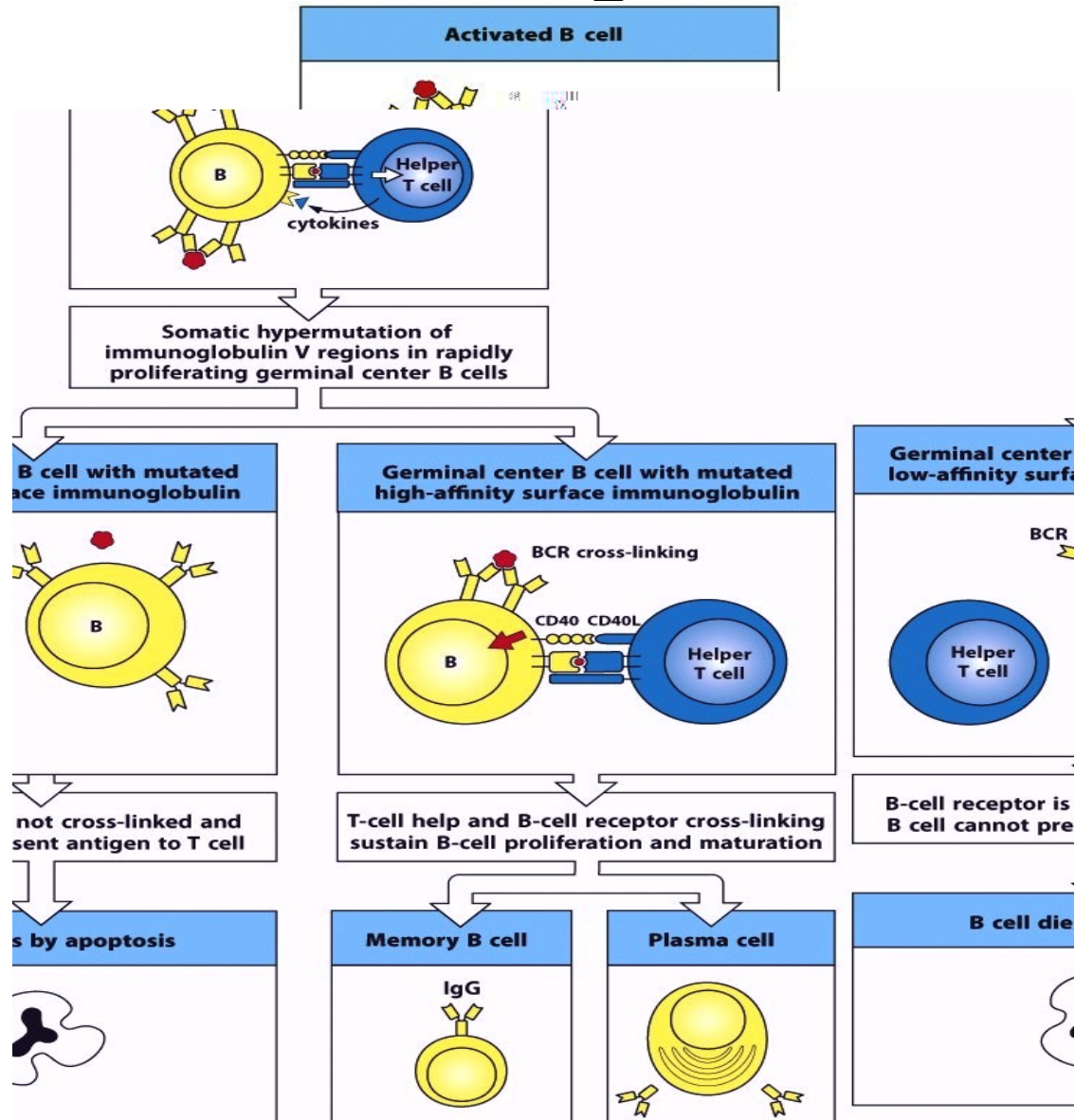


Figure 10.13 Janeway

Class switching

- Mediated by T_{FH} in the light zone.
- They interact with B-cells through surface molecules, and they produce cytokines resulting in class switch recombination.
- As a result, B cells switch to synthesizing Ig of a different isotype: IgG, IgA or IgE aided by the enzyme AID in GC.
- The cytokine produced by the T_{FH} cell influences the antibody isotype synthesized by the B cell: IL-4 induces IgE and IgG4 synthesis; IFN- γ induces IgG3 synthesis.

Somatic hypermutation vs. class switch

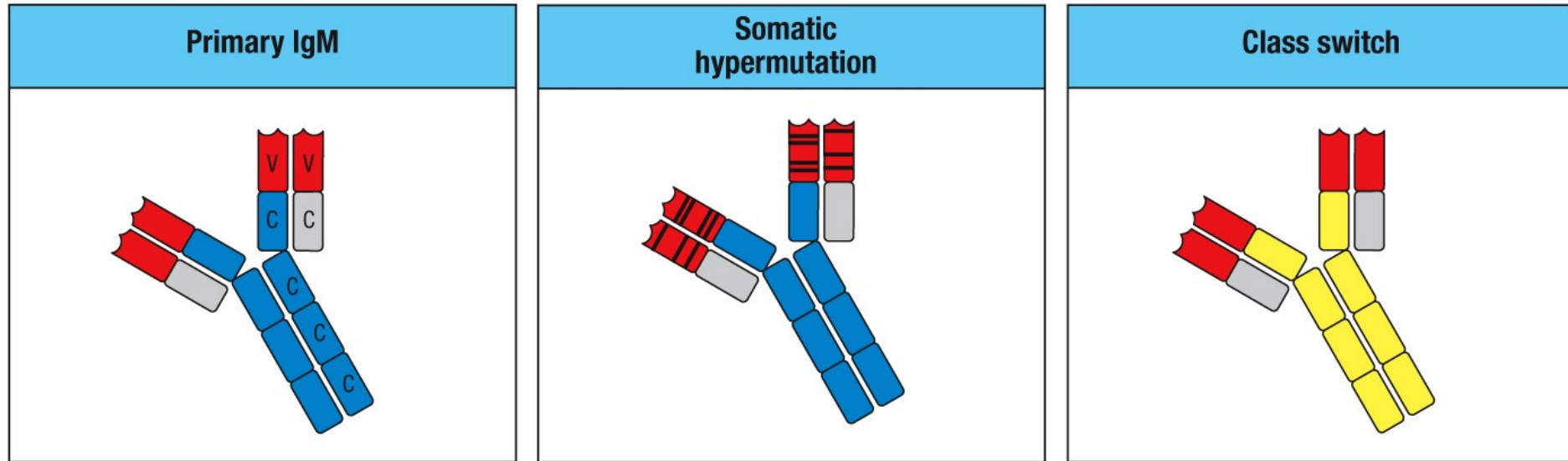
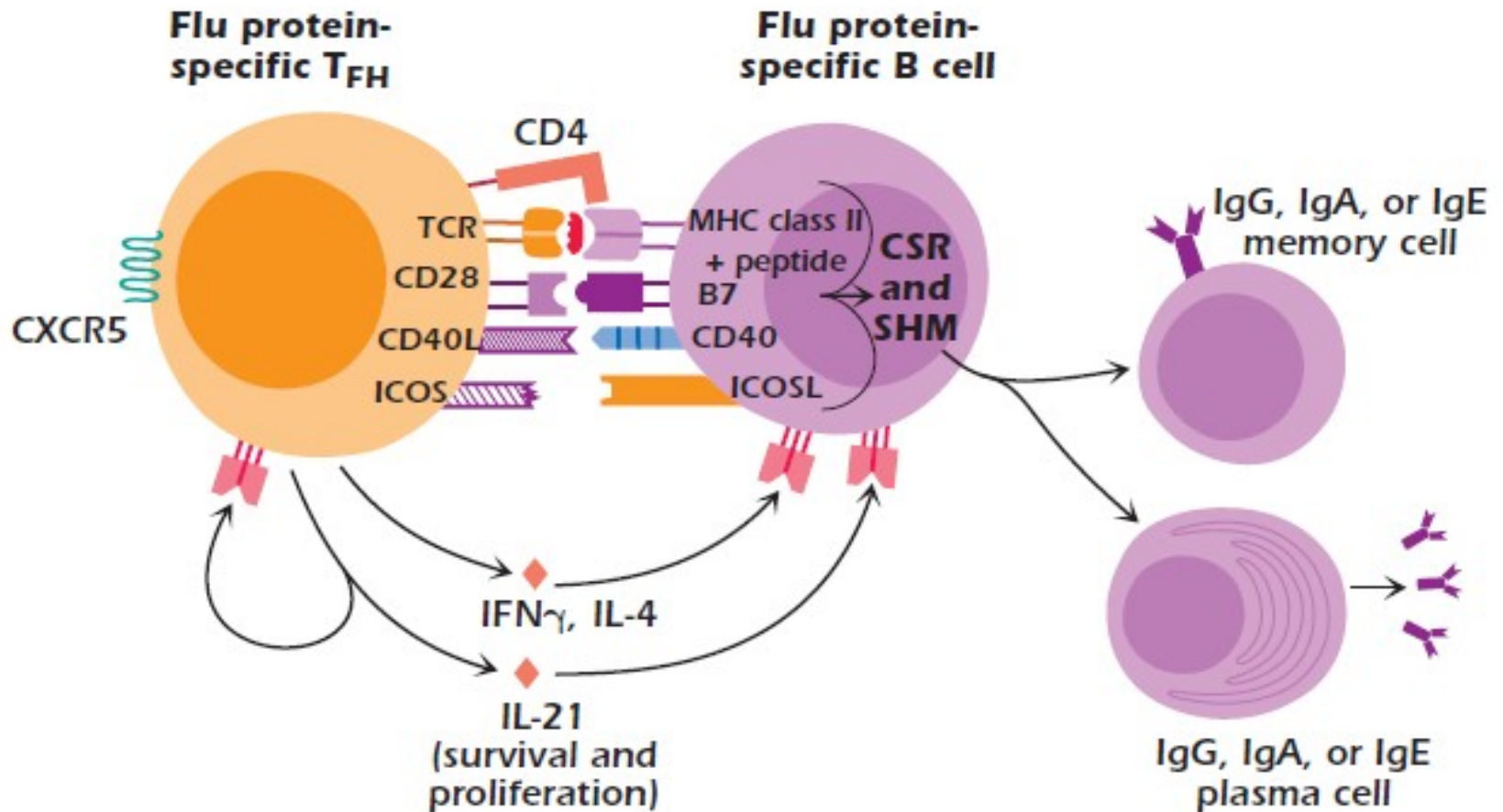


Figure 10.13 Janeway's Immunobiology, 9th ed. (© Garland Science 2017)

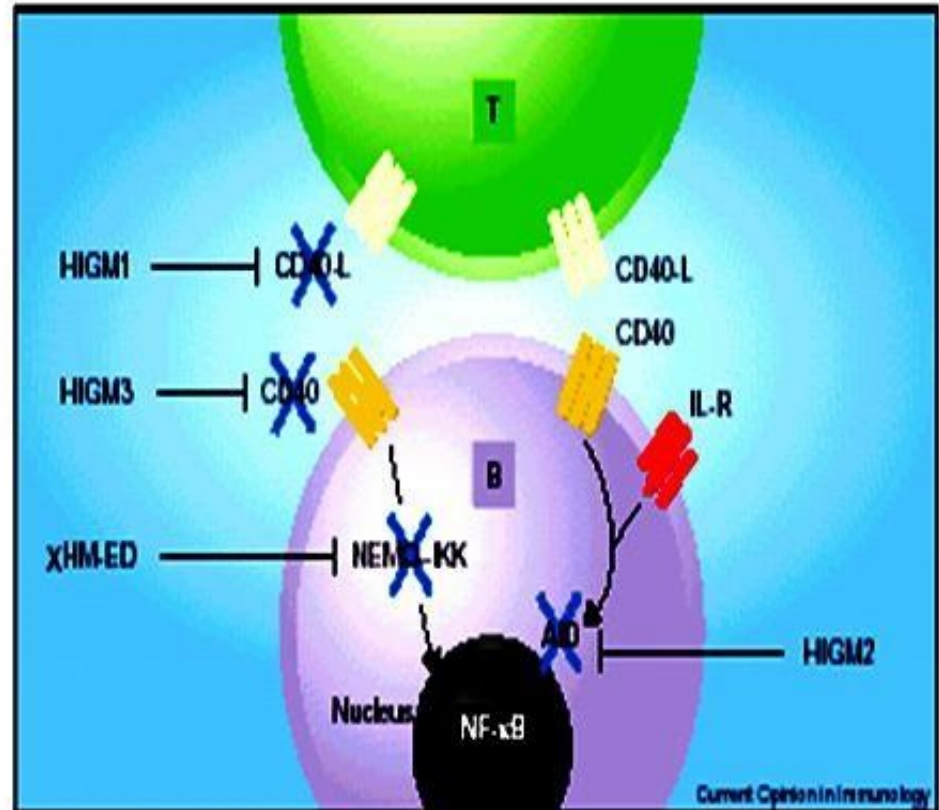
GC events



	Intrinsic properties			Inducible by antigen stimulation		
B-lineage cell	Surface Ig	Surface MHC class II	High-rate Ig secretion	Growth	Somatic hyper-mutation	Class switch
 Resting B cell	High	Yes	No	Yes	Yes	Yes
 Plasmablast	High	Yes	Yes	Yes	Unknown	Yes
 Plasma cell	Low	No	Yes	No	No	No

Hyper-IgM syndromes

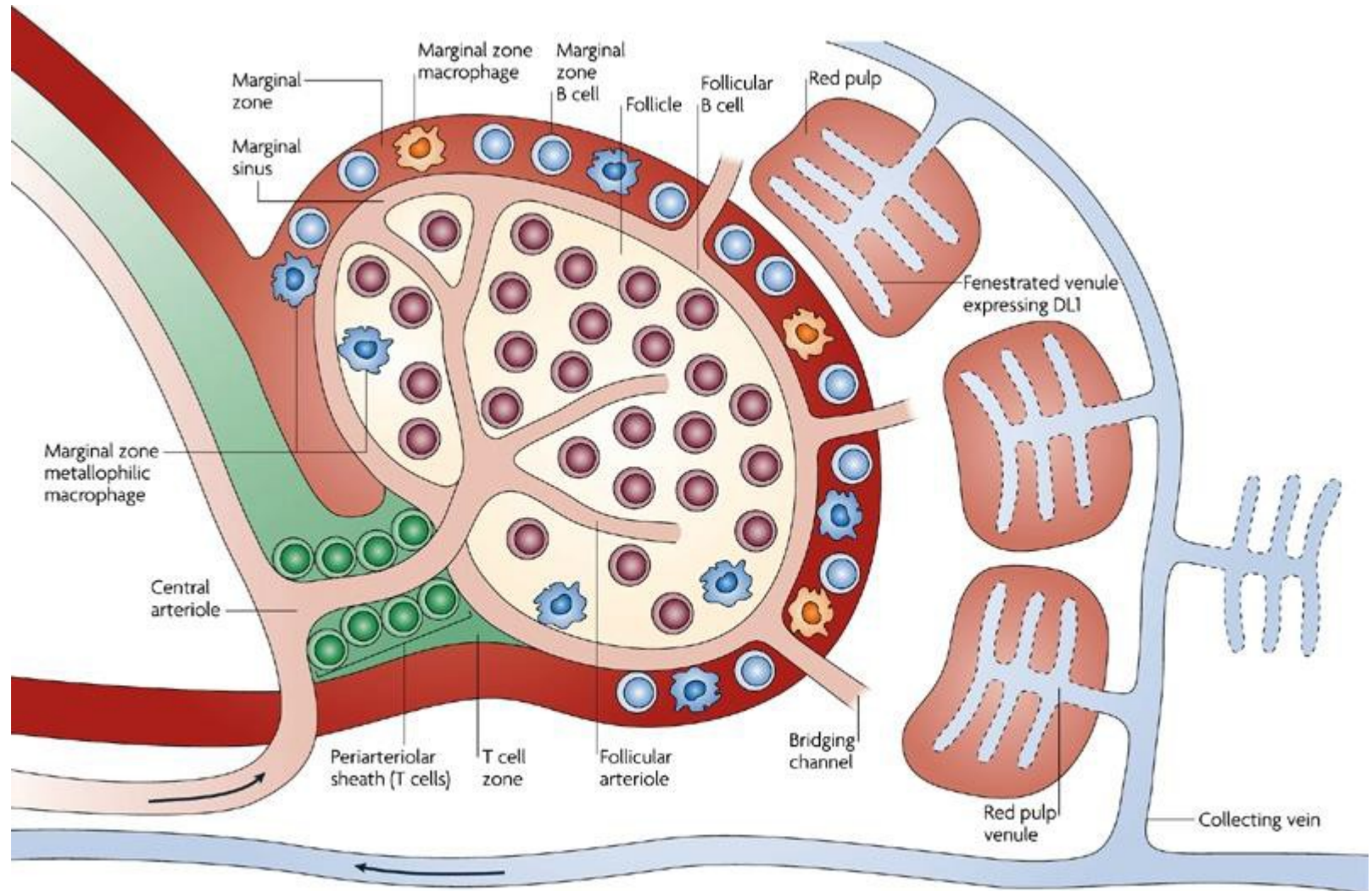
- Deficiency in the activity of AID or from mutations in the expression of surface molecules by follicular B or Tfh.
- Inability to provide help to B cells.
- Hence high serum IgM, failure to switch to IgG, IgA, IgE.
- No germinal centres.
- No memory B-cells.



Marginal Zone B cells

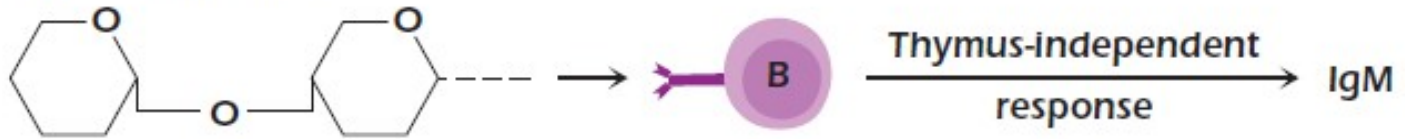
- The marginal zone of the spleen separates the regions containing T and B lymphocytes from the red pulp.
- Contains macrophages that filters blood-borne antigens.
- Also contains a distinct population of mature, long-lived B-cells — marginal zone B-cells (differs from follicular B-cells).
- Function: Tl synthesis of IgM in response to blood-borne or lymph-borne pathogens, e.g. encapsulated bacteria (polysaccharide coated) and viruses.
- Children <2 years lack well developed marginal zone B cells.

Marginal Zone B cells

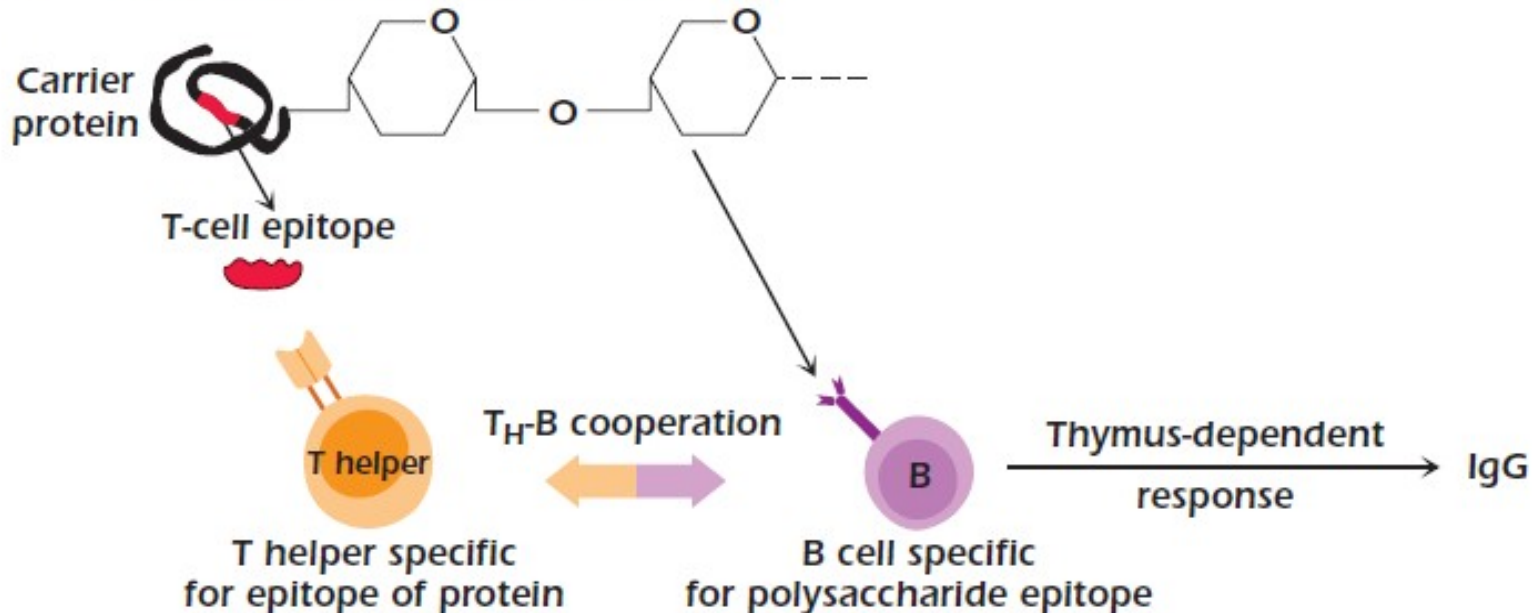


B cell activation in response to TI antigens: conjugate vaccines.

A Polysaccharide alone



B Protein conjugated to polysaccharide



CD8+T cells

- Referred to as a **killer T cell** or **cytotoxic T lymphocyte** (CTL).
- They kill cells infected by viruses and bacteria and involved in killing of transplanted foreign cells (graft rejection) and tumour cells.
- Responses are initiated after the virus is transported to the lymph node draining the infected organ or tissue, where it is taken up by macrophages or DCs.

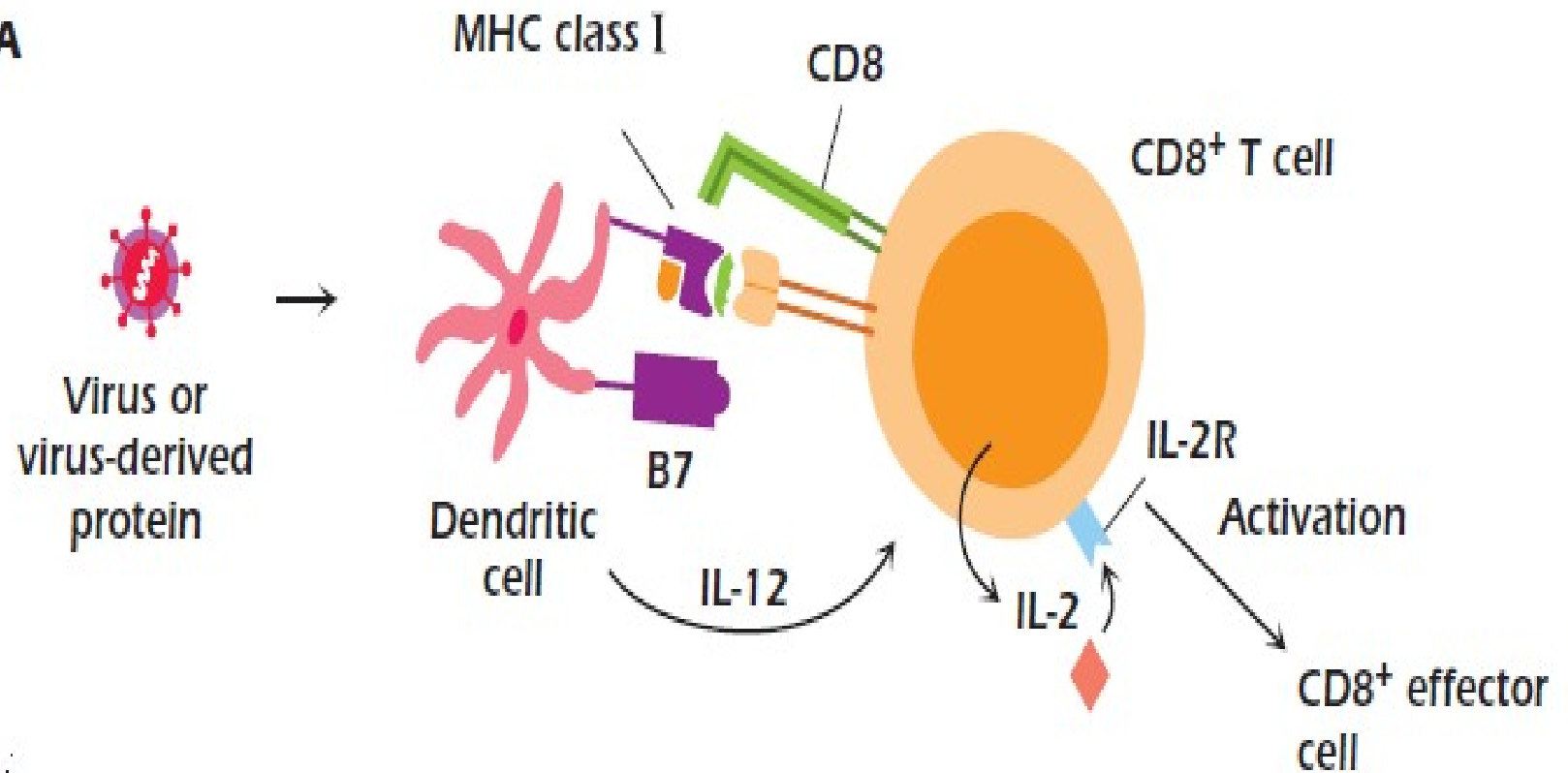
CD8+T cells activation

Two pathways for CD8+ T cells activation:

- **First pathway:** DC present viral peptides through MHC I to cytotoxic T cells.
- DCs provide the first and second signals, as well as **IL-12**, to directly activate the CD8+ T cell.
- These signals induce proliferation CD8+ T cell which synthesize IL-2 that in turn induces the proliferation of the activated CD8+ T cells.

CD8⁺T cells activation

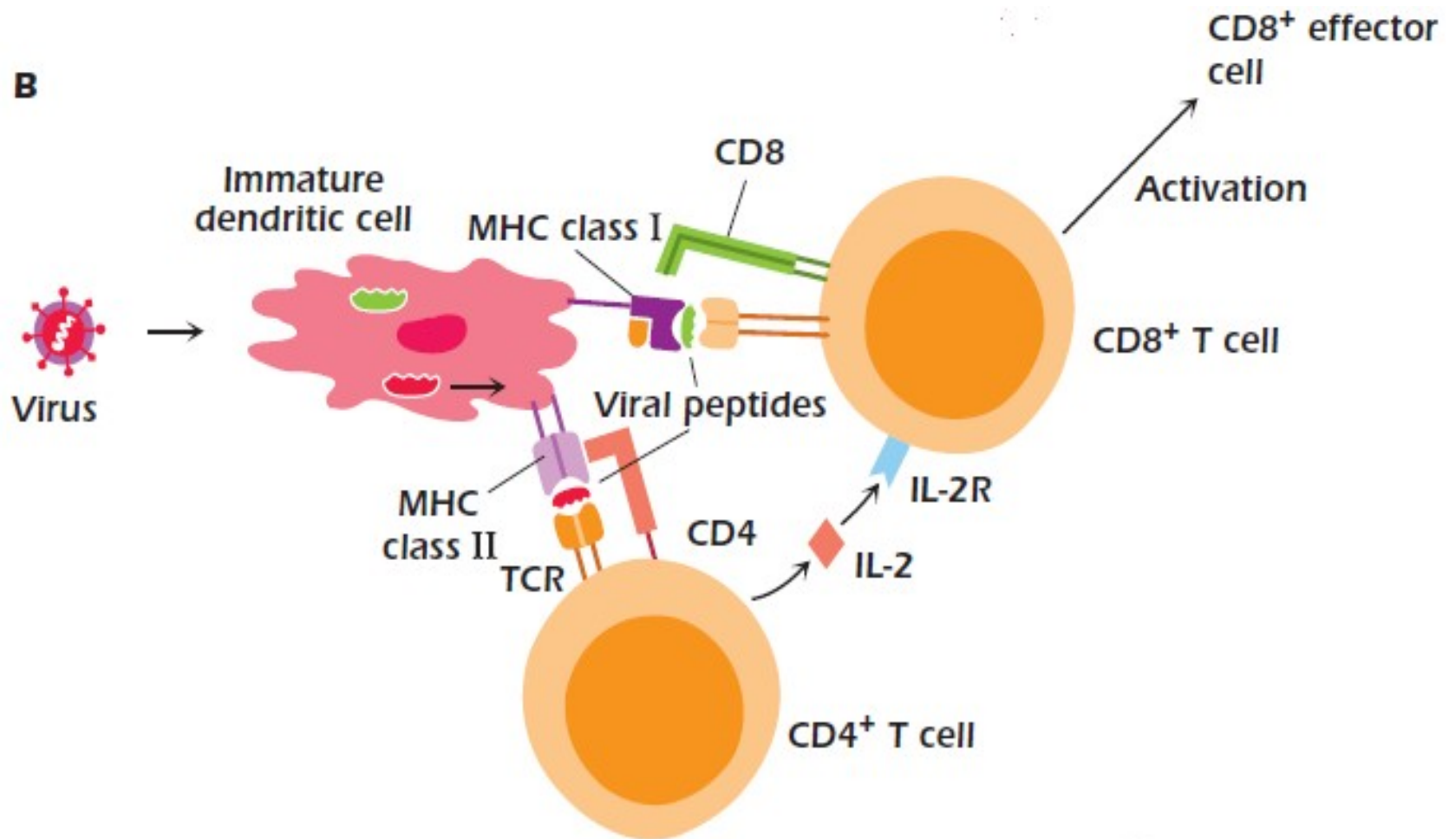
A



CD8+T cells activation

- **Second pathway:** CD8+ T cells activation requires help from virus-specific CD4+ T cells.
- A DC takes up virus and presents virus-derived peptides in association with MHC II molecules to a virus specific CD4+ T.
- The same DC also present virus antigens to CD8+ T cells via MHC I molecules.
- IL-2 synthesized by the CD4+ T cell, induces virus-specific CD8+ T-cell proliferation and differentiation.
- DC can be interacting with CD4+ T cells and CD8+ T-cell at the same time or in separate events.

CD8+T cells activation



CD8+T cells activation

- The same pairs of co-stimulator and adhesion molecules employed in the activation of CD4+ T cells are also involved in CD8+ cells activation.
- Whichever pathway is used to activate the CD8+ T cell, the intracellular events in CD8+ T-cell activation are similar to those for CD4+ T-cell activation.
- The negative co-stimulator molecules CTLA-4 and PD1 are also expressed on CD8+ T cells: they turn the immune response off.

CD8+T cells killing

- Activated CD8+ T cells initiate killing by attaching to the target cell.
- The infected target expresses MHC class I and virus-derived peptides.
- CD8+ T binds MHC I on infected cell through its TCR and activate apoptosis of the infected cell through two pathways:
 1. By the action of the granule contents of activated CD8+ T cells.
 2. Through Fas-Fas ligand cell surface interactions.

CD8+T cells killing

First Pathway

- The predominant pathway for killing most target cells.
- Involves the release of granules contents of CD8+ T cells by exocytosis: granules that formed when the CD8+T cell is activated.
- The granules contain:
 - **Perforin:** forms pores in the target cell membrane increasing its permeability.
 - **Granzymes:** proteases that pass into the target cell through the pores created by perforin and induce apoptosis.
 - **Granulysin:** activates pathways involved in killing of intracellular bacteria such as *Listeria* and *Mycobacteria*, which live inside macrophages and DCs.

CD8+T cells killing

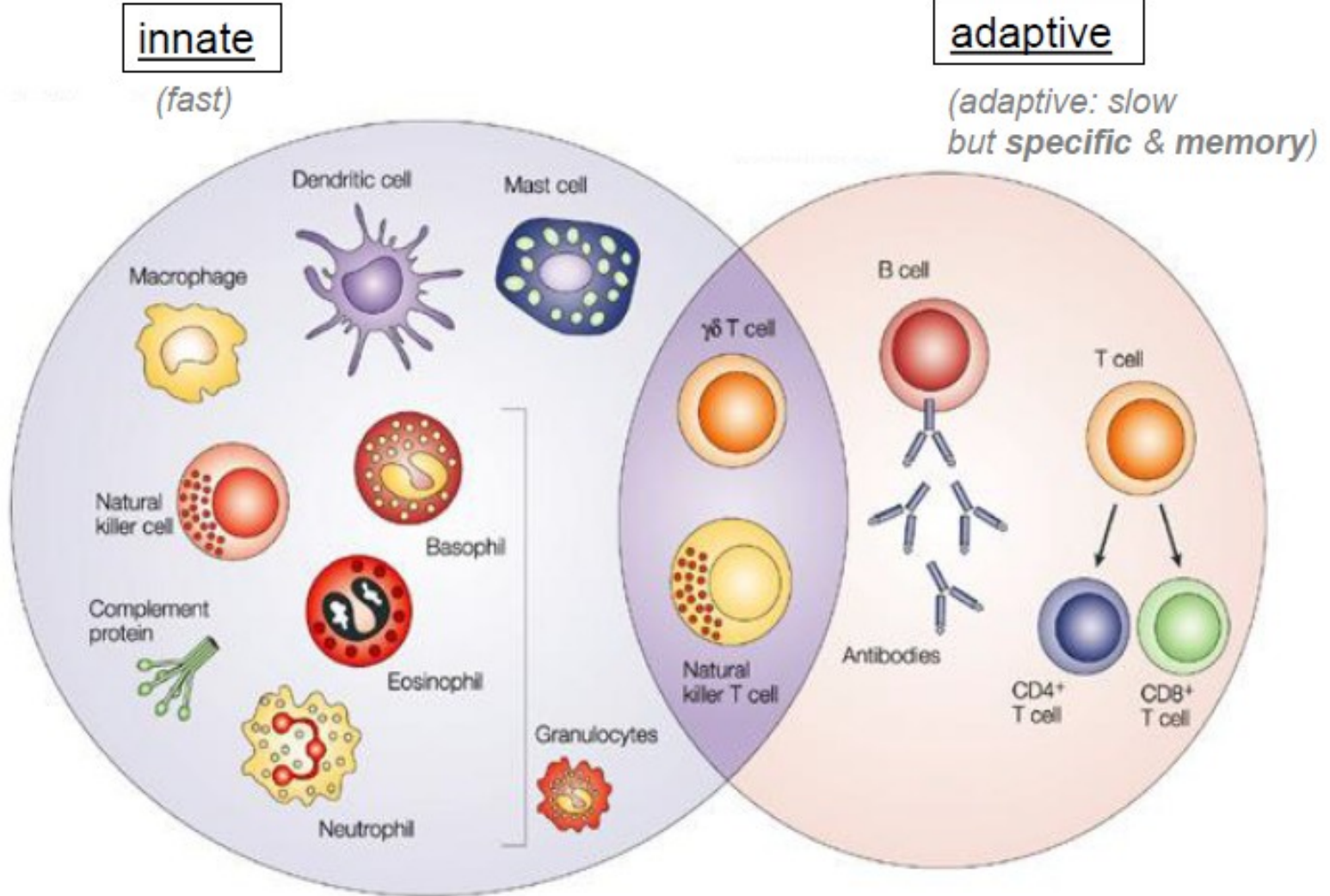
Second pathway

- Target cell killing occurs via the interaction of the molecule **FasL** expressed on the CD8+ T cell, with **Fas**, expressed on many host cells.
- This interaction activates the apoptosis of the target cell via sequential activation of **caspases** —proteolytic enzymes, inside the target cell.

Memory T cells

- A small population of the activated CD4+ and CD8+ T cells become memory cells.
- They are long-lived and are involved in protective responses after a subsequent exposure to the same pathogen — Memory T-cell responses are also more rapid and effective than primary responses.
- **Two types:**
 1. Effector memory cells: reside in tissues e.g. skin and mucosa
 2. Central memory cells: recirculate through SLO, develop into effector cells.

The Immune system:



Complement



DR. DALIA WEHEDI

History



- Research on complement began in the 1890s, when Jules Bordet at the Institute Pasteur in Paris showed that sheep antiserum to the bacterium *Vibrio cholerae* caused lysis of the bacteria and that heating the antiserum destroyed its bacteriolytic activity.
- He named those substances Alexins.
- Paul Ehrlich coined the term complement.



Introduction



- It is named “complement system” because it was first identified as a heat-labile component of serum that “complemented or augment” antibodies in the killing of bacteria
- Consists of serum and cell surface proteins involved in defense against pathogens and tissue damage mediated by antibodies
- The Complement system is the major effector of cellular and humoral branch of immune system.
- Plays major role in both innate and adaptive immunity

Complement system



- Complement system represents a group of proteins which augment or complement the immune response.
- Most of these proteins are found in serum or on cell surfaces.
- Synthesized in liver as inactive precursors and are activated by proteolysis during their interaction in a sequential manner.

Complements



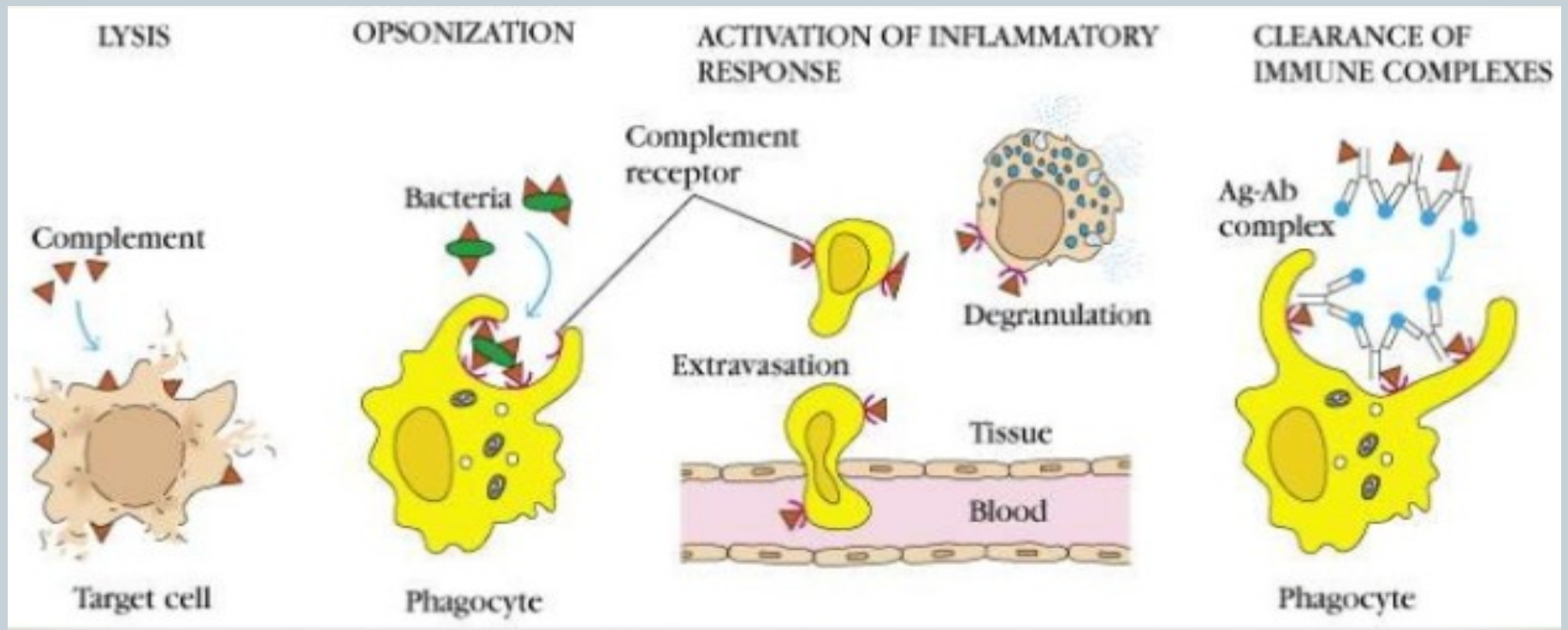
- Complement of one species are able to react with antibodies of other species but not to the same extent.
- C- proteins constitute about 3-5% of normal serum protein
- Are glycoproteins.
- Are synthesized rapidly in inflammatory responses – hence are called acute phase proteins.
- Heat labile and lost activity at 56°C for 30 mins. and inactivated. Immunoglobulins are not inactivated at this temperature.
- Binds with Fc portion of immunoglobulins .

Complements



Three main effects of complement are:

1. Lysis of cells (bacteria, allografts, tumor cells)
2. Generation of mediators of inflammation
3. Opsonization – enhancement of phagocytosis



Complements



- Over 30 serum and cell surface proteins.
- Complement components:

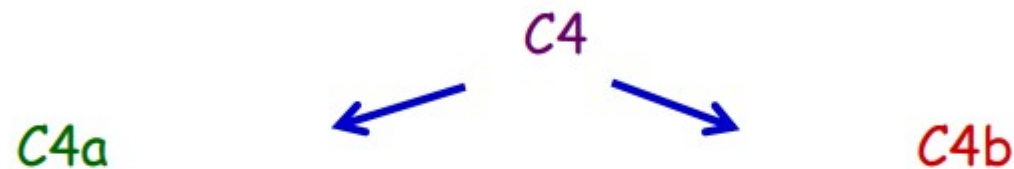
Components are designated by numbers (E.g. ; C1 – C9) or letters (E.g. : Factor D). In serum inactive, activated sequentially as a cascade..

- Complement receptors
cell surface, recognize activated components
- Regulatory proteins of complement
both in serum and cell surface, inhibit activated components



- Complement proteins: are proenzymes - activation by cleavage.

- Example:



a = smaller fragment.
-Diffusion

b= larger fragment.
-remains bound to microbe

- Exception: C2: C2a = large fragment
C2b = small fragment

Complement pathways



1. Classical pathway:-

Is antibody dependent pathway and triggered by formation of soluble antigen-antibody complex or by binding of the antibody to the antigen present on the target cell surface.

2. Alternative pathway:-

Is antibody independent pathway stimulated by antigen directly eg. Bacterial cell surface components.

3. Lectin Pathway:-

Also antibody independent but resembles classical pathway.

Stages of complement Activation



Three main stages in the activation of complement by any pathway are

- Formation of C3 convertase
- Formation C5 convertase
- Formation of membrane attack complex (MAC)

The initiation and formation of C3 convertase are different in classical and alternative pathway . These then follow the parallel route to merge at C5 convertase stage and finally generate the MAC by a common routes



- Sequential activation of complement components occurs via one of three pathways:
 - ▢ the classic pathway,
 - ▢ the lectin pathway, and
 - ▢ the alternative pathway .
- Of these pathways, the lectin and the alternative pathways are more important the first time we are infected by a microorganism because the antibody required to trigger the classic pathway is not present.
- The lectin pathway and the alternative pathway are, therefore, participants in the innate arm of the immune system.



- All three pathways lead to the production of C3b, the central molecule of the complement cascade.
- The presence of C3b on the surface of a microbe marks it as foreign and targets it for destruction. C3b has two important functions:
 - (1) It combines with other complement components to generate C5 convertase, the enzyme that leads to the production of the membrane attack complex and
 - (2) It opsonizes bacteria because phagocytes have receptors for C3b on their surface.

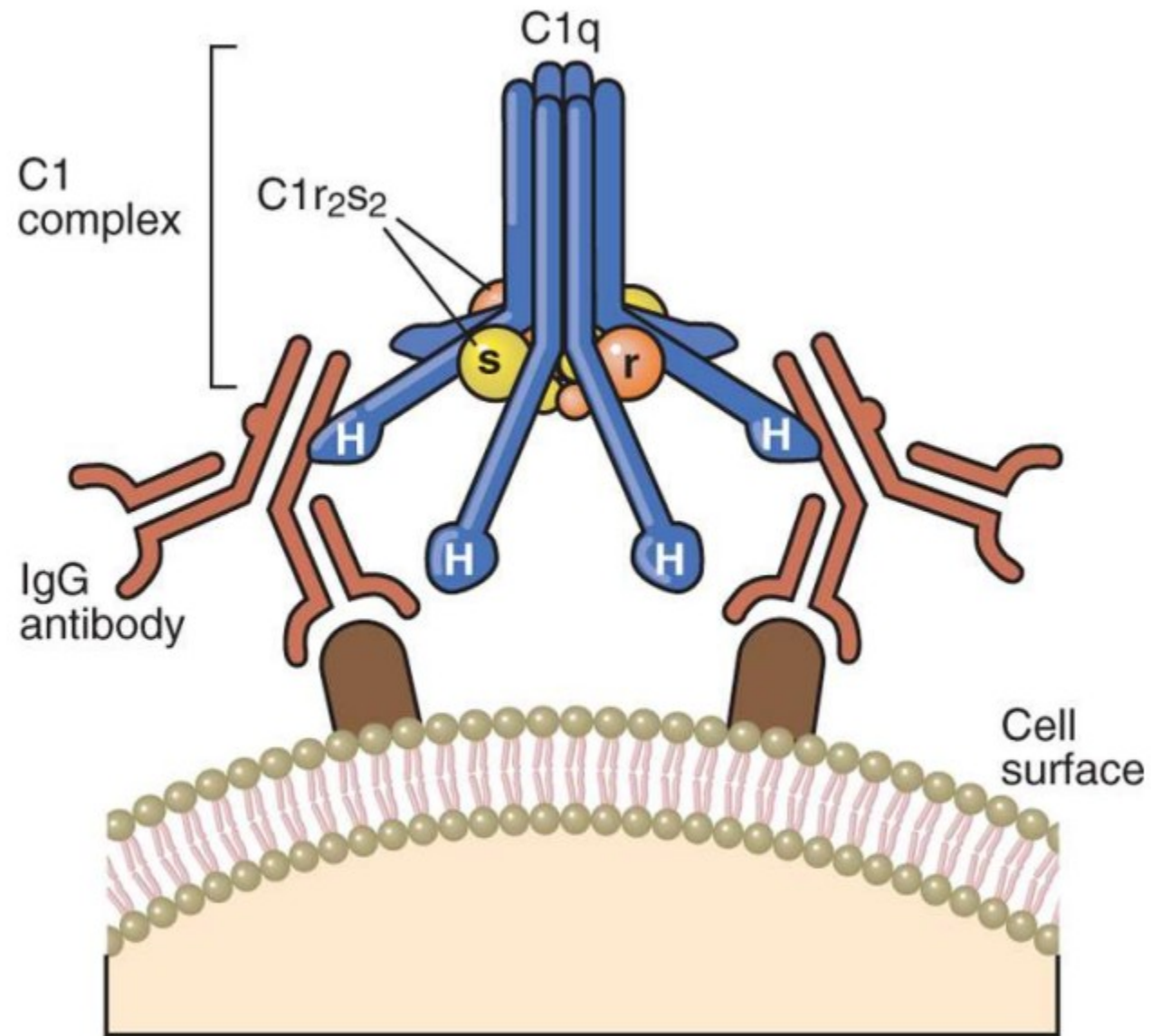
Classical pathway



- In the classic pathway, antigen–antibody complexes activate C1 to form a protease, which cleaves C2 and C4 to form a C4bC2a complex, C2a and C4b split off.
- The C4bC2a is C3 convertase, which cleaves C3 molecules into two fragments, C3a and C3b.
- C3b forms a complex with C4b,2b, producing a new enzyme, C5 convertase (C4b2a3b), which cleaves C5 to form C5a and C5b
- C5b binds to C6 and C7 to form a complex that interacts with C8 and C9 to produce the membrane attack complex (C5b,6,7,8,9), which causes cytolysis.
- Note that the "b" fragment continues in the main pathway, whereas the "a" fragment is split off and has other activities

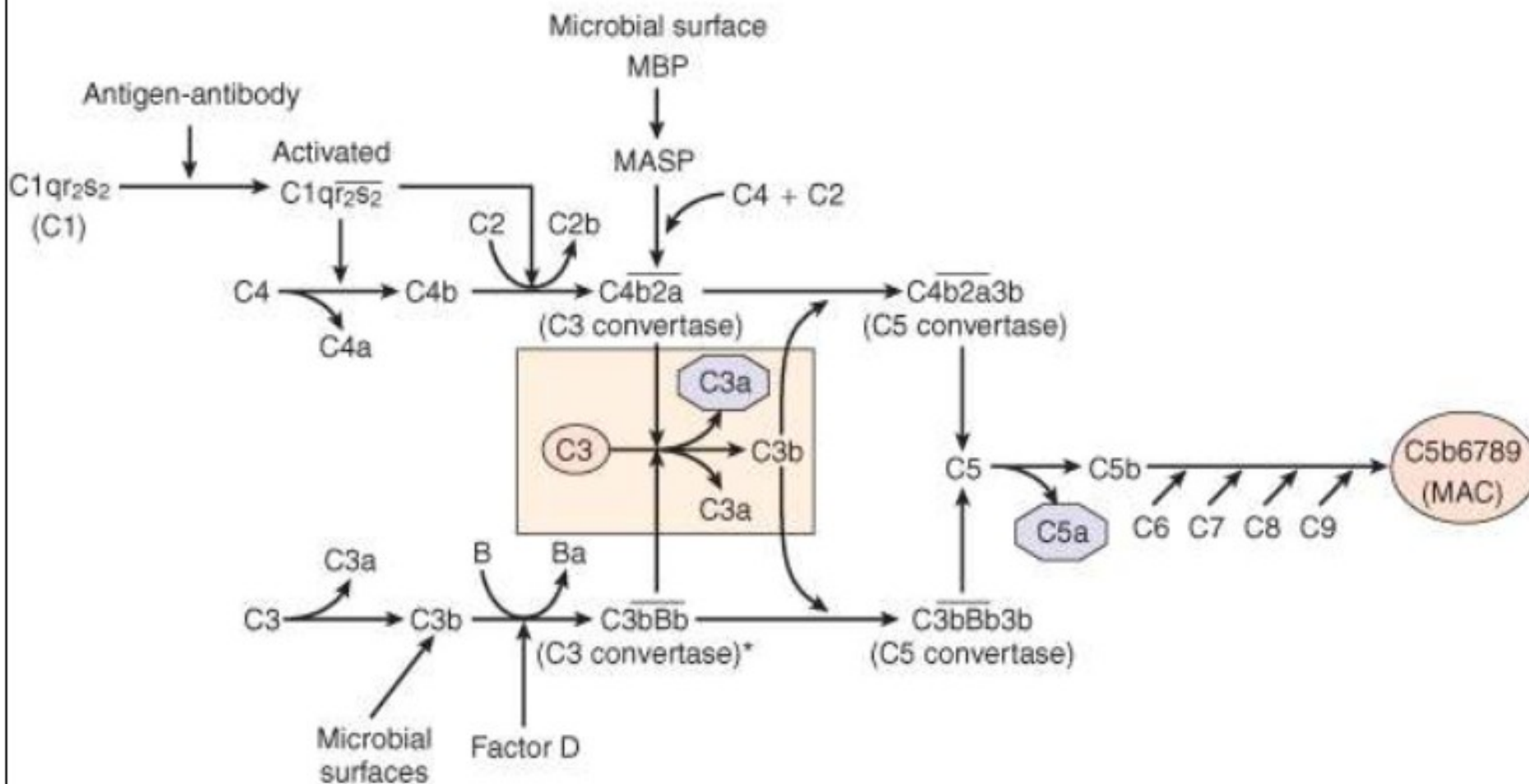


- Only IgM and IgG fix complement.
- One molecule of IgM can activate complement; however, activation by IgG requires two cross-linked IgG molecules.
- C1 is bound to a site located in the Fc region of the heavy chain.
- C1 is composed of three proteins, C1q, C1r, and C1s.
- C1q is an aggregate of 18 polypeptides that binds to the Fc portion of IgG and IgM.
- It is multivalent and can cross-link several immunoglobulin molecules.
- C1s is a proenzyme that is cleaved to form an active protease



Classical Pathway

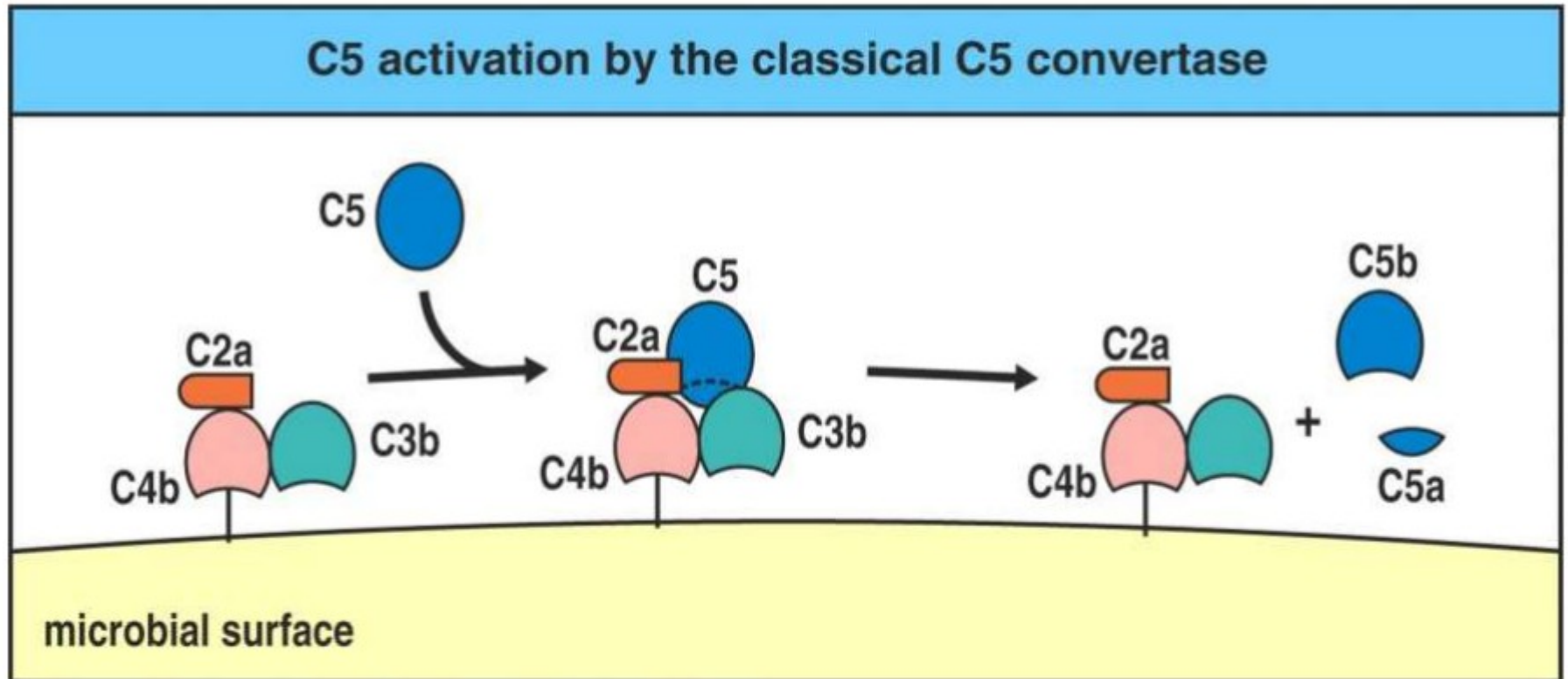
Lectin Pathway



Alternate Pathway

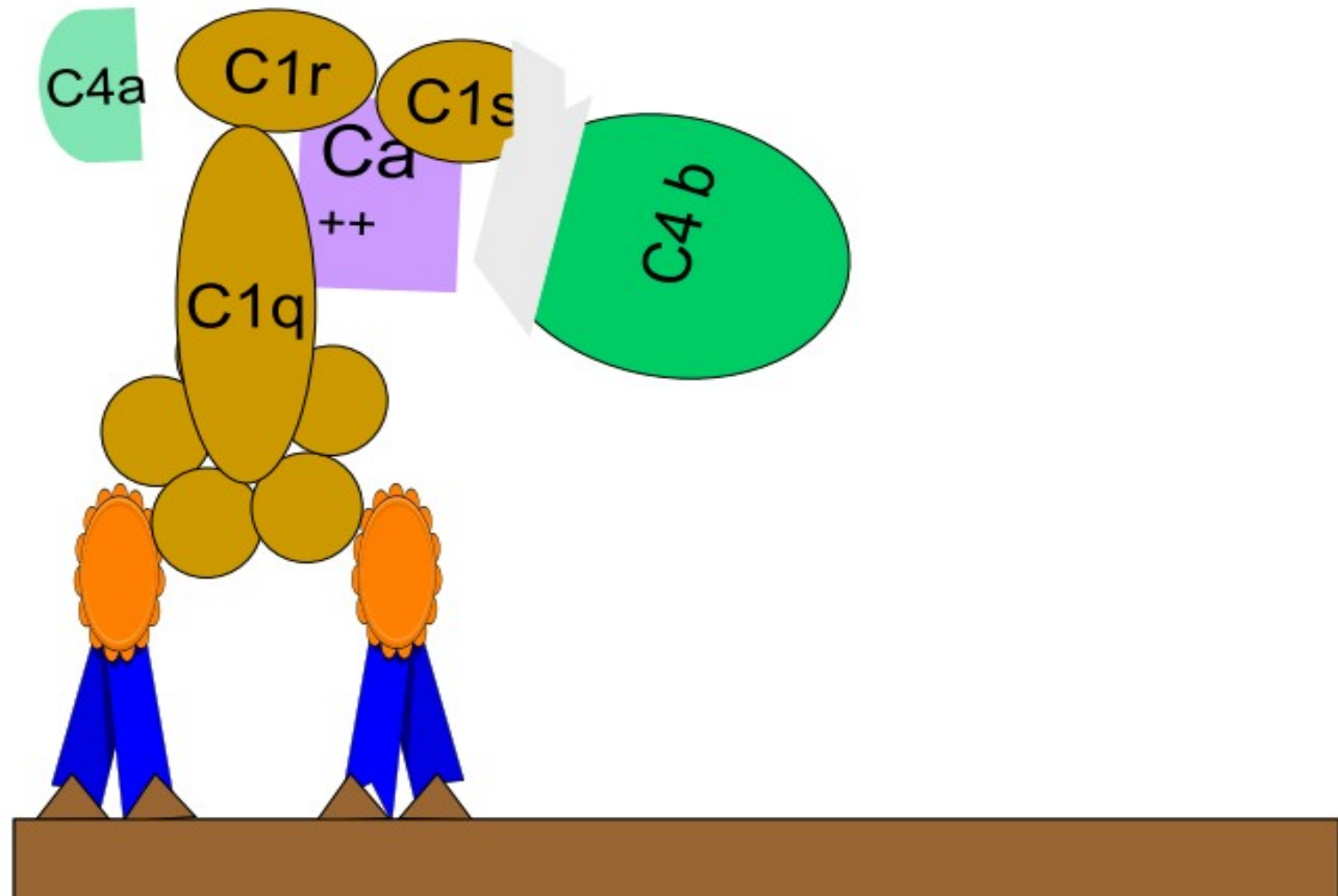
* Stabilized by properdin

C5 activation



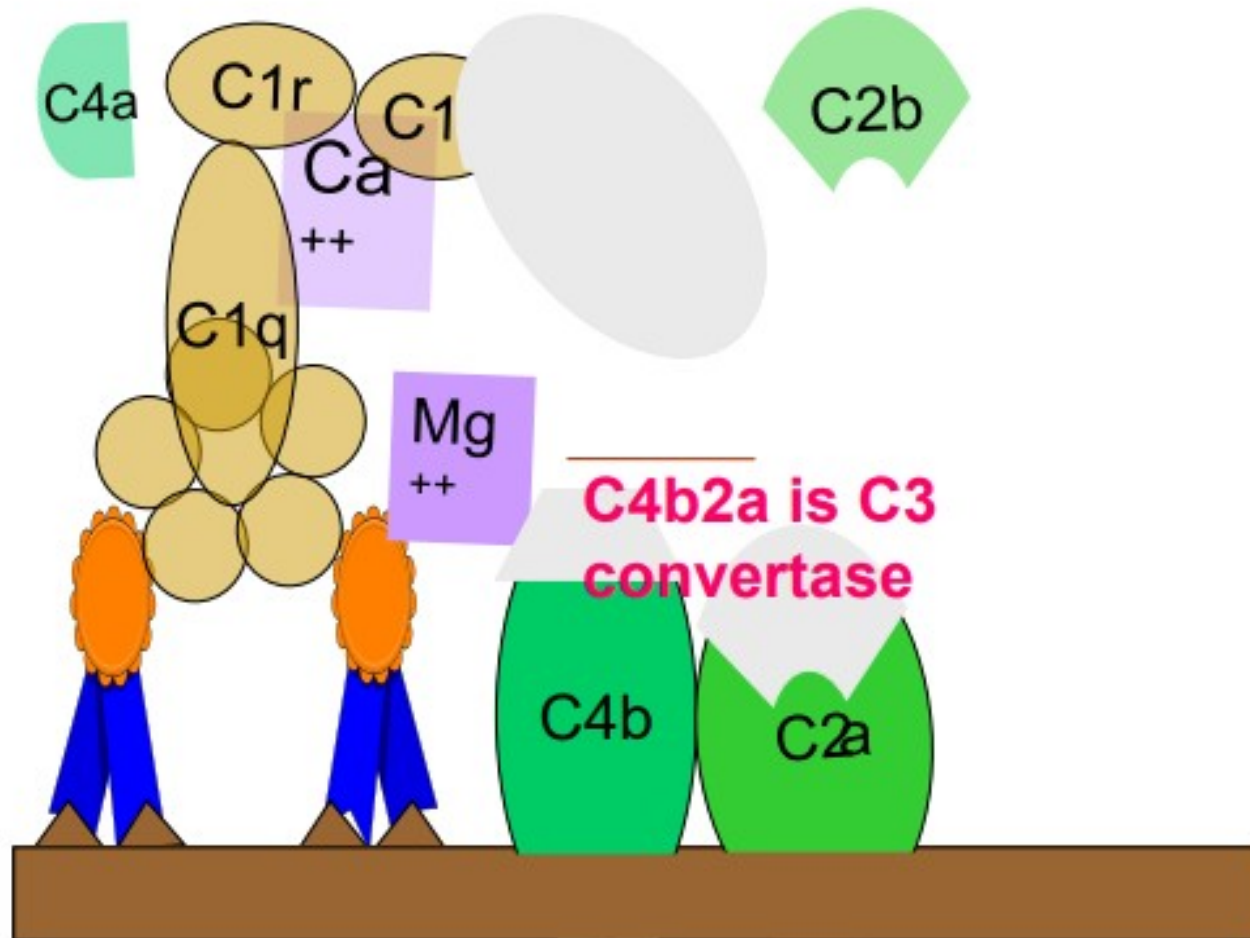
- C4b-2a-3b functions as the classical C5 convertase:

Classical Pathway Generation of C3-convertase

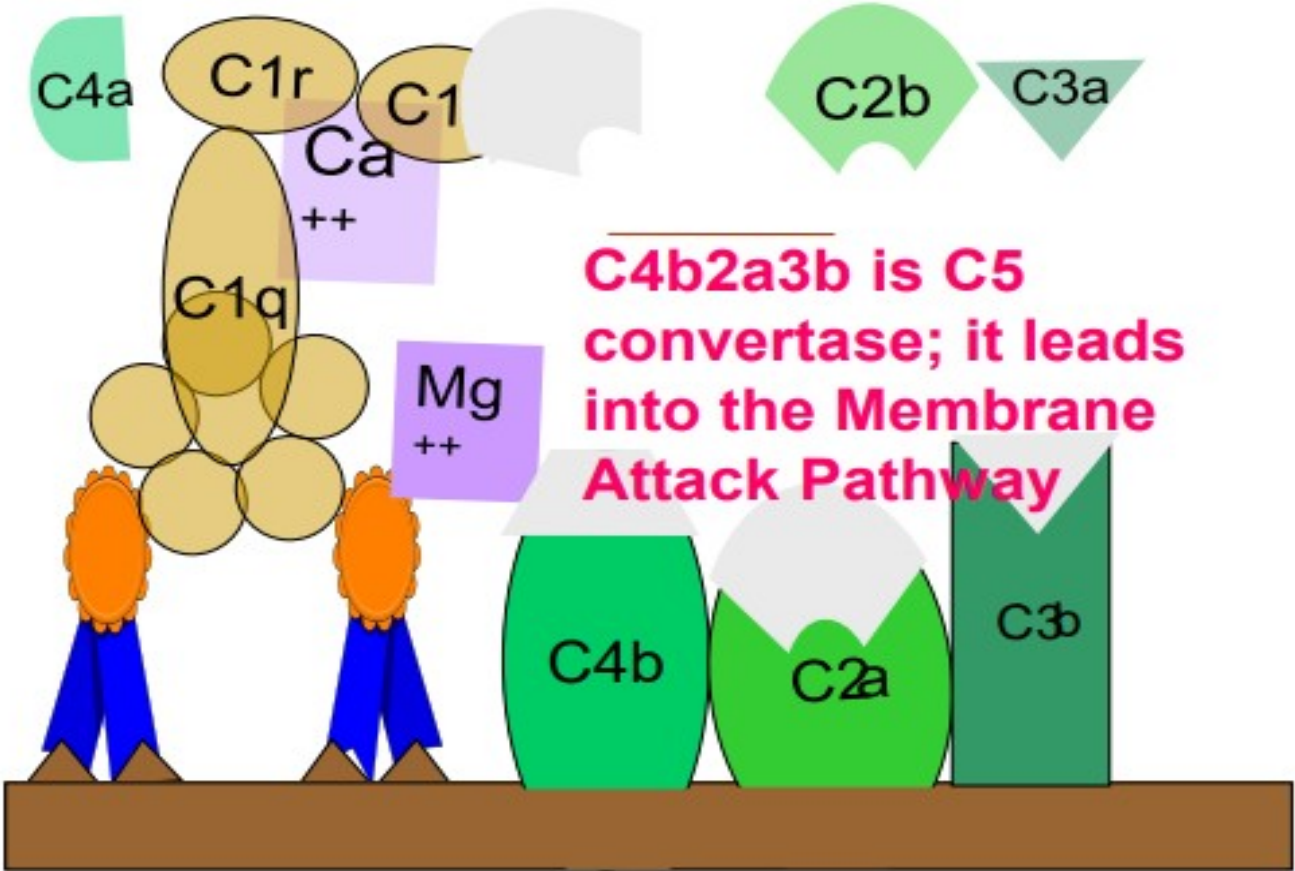


Classical Pathway

Generation of C3-convertase



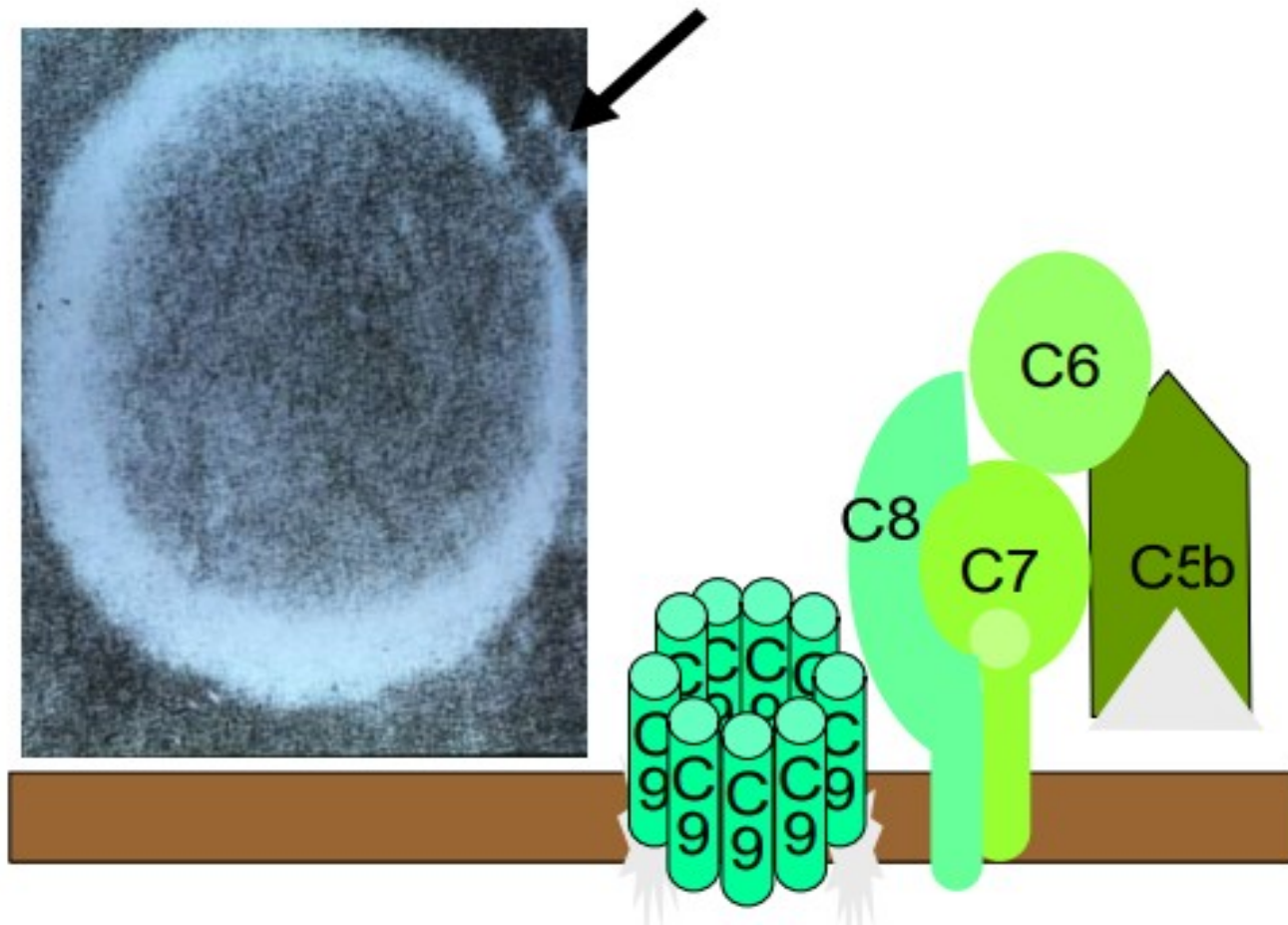
Classical Pathway Generation of C5-convertase



Lytic pathway assembly of the lytic complex



Lytic pathway:
insertion of lytic complex into cell
membrane



Classical pathway

Components of the Classical Pathway

Native component	Active component(s)	Function(s)
C1(q,r,s)	C1q	Binds to antibody that has bound antigen, activates C1r.
	C1r	Cleaves C1s to activate protease function.
	C1s	Cleaves C2 and C4.
C2	C2a	Unknown.
	C2b	Active enzyme of classical pathway; cleaves C3 and C5.
C3	C3a	Mediates inflammation; anaphylatoxin.
	C3b	Binds C5 for cleavage by C2b. Binds cell surfaces for opsonization and activation of alternate pathway.
C4	C4a	Mediates inflammation.
	C4b	Binds C2 for cleavage by C1s. Binds cell surfaces for opsonization.

Classical pathway



Components of the Membrane-Attack Complex

Native component	Active component(s)	Function(s)
C5	C5a	Mediates inflammation; anaphylatoxin, chemotaxin.
	C5b	Initiates assembly of the membrane-attack complex (MAC).
C6	C6	Binds C5b, forms acceptor for C7.
C7	C7	Binds C5b6, inserts into membrane, forms acceptor for C8.
C8	C8	Binds C5b67, initiates C9 polymerization.
C9	C9n	Polymerizes around C5b678 to form channel that causes cell lysis.

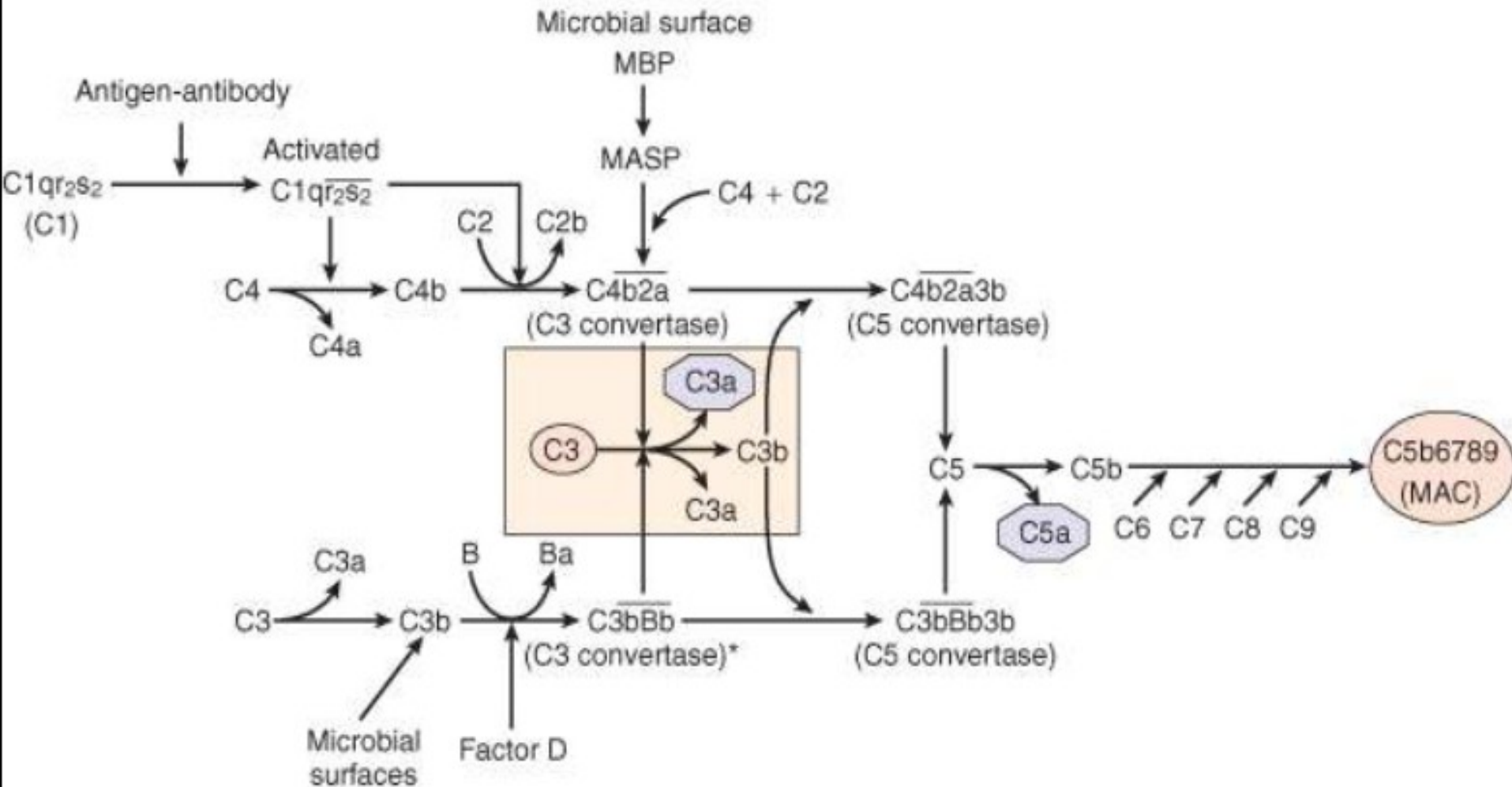
Alternative pathway



- Ab independent pathway.
- In the alternative pathway, many unrelated cell surface substances, e.g., bacterial lipopolysaccharides (endotoxin), fungal cell walls, and viral envelopes, can initiate the process by binding C3 and factor B.
- This complex is cleaved by a protease, factor D, to produce C3bBb.
- This acts as a C3 convertase to generate more C3b.

Classical Pathway

Lectin Pathway



Alternate Pathway

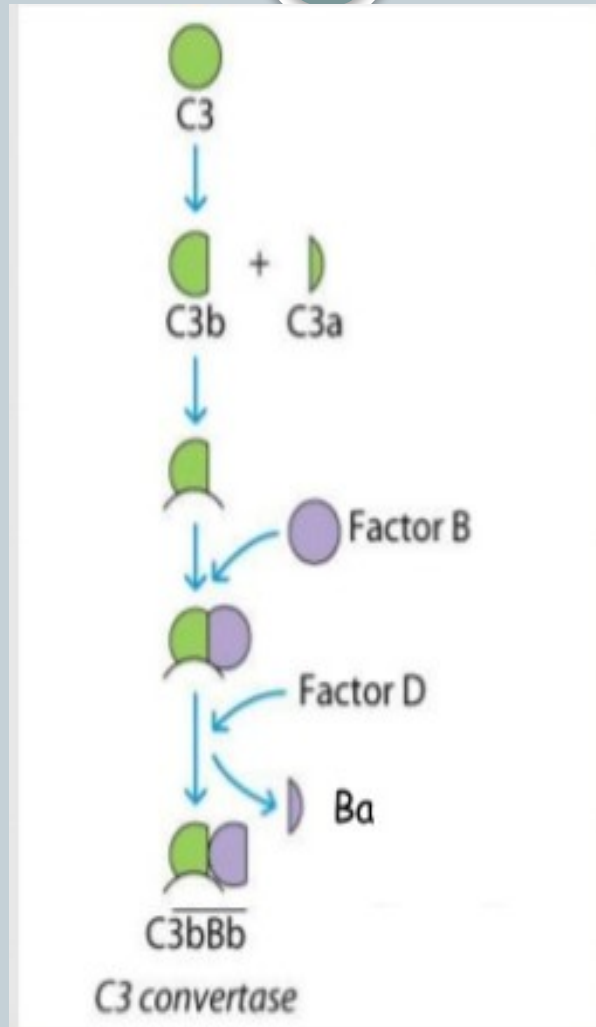
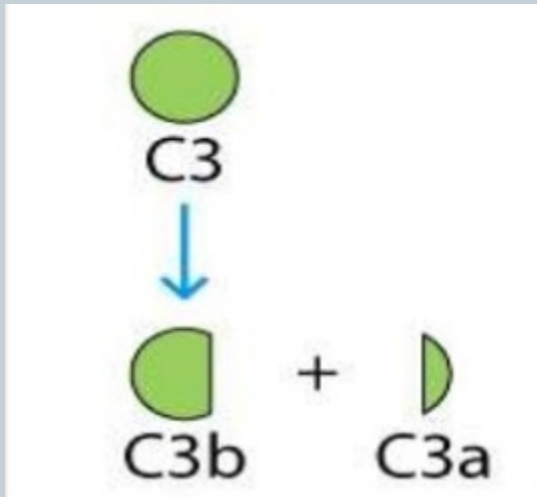
* Stabilized by properdin

Alternative Pathway

Components of the Alternate Pathway

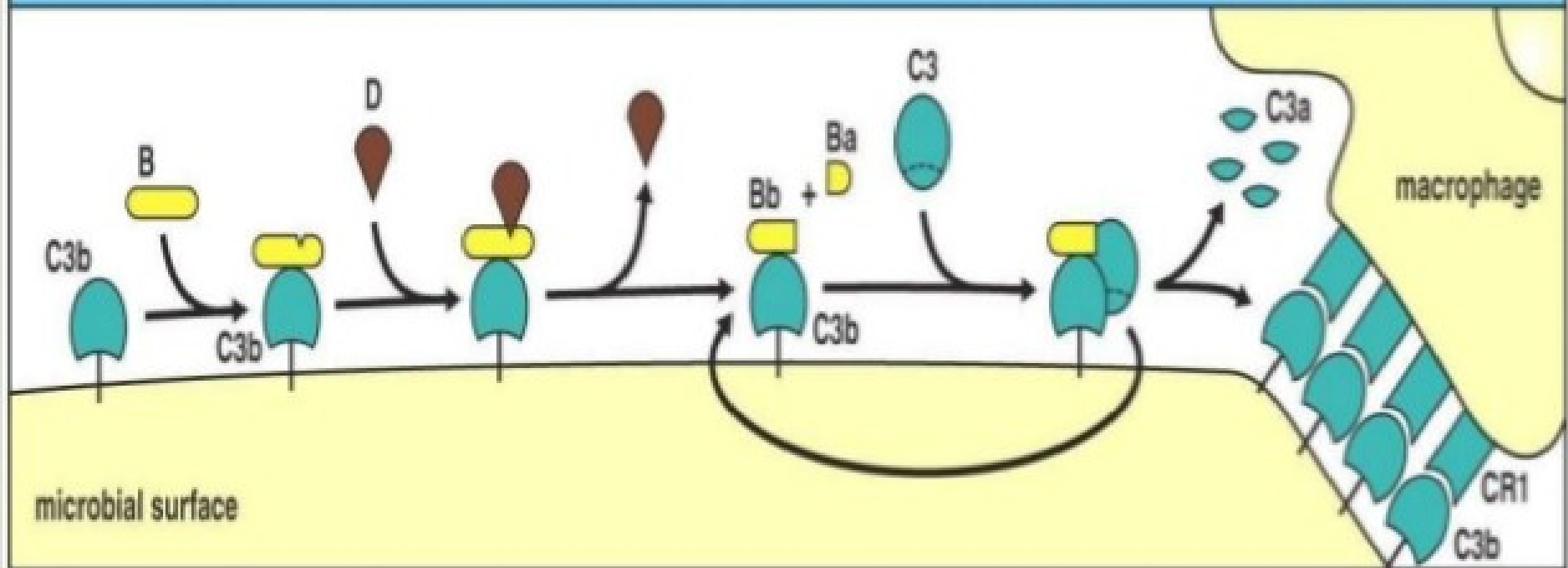
Native component	Active component(s)	Function(s)
C3	C3a	Mediates inflammation; anaphylatoxin.
	C3b	Binds cell surfaces for opsonization and activation of alternate pathway.
Factor B	B	Binds membrane bound C3b. Cleaved by Factor D.
	Ba	Unknown.
	Bb	Cleaved form stabilized by P produces C3 convertase.
Factor D	D	Cleaves Factor B when bound to C3b.
Properdin	P	Binds and stabilizes membrane bound C3bBb.

Alternative pathway



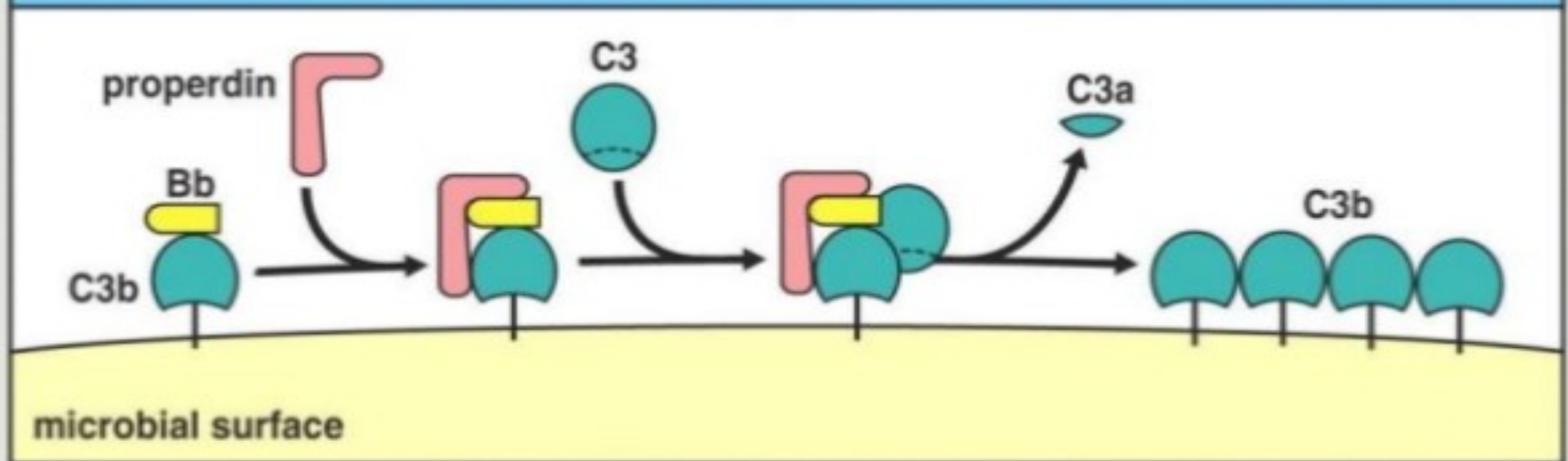
Alternative pathway

Formation and action of the C3 convertase C3bBb of the alternative pathway at a microbial surface



Properdin- Factor P

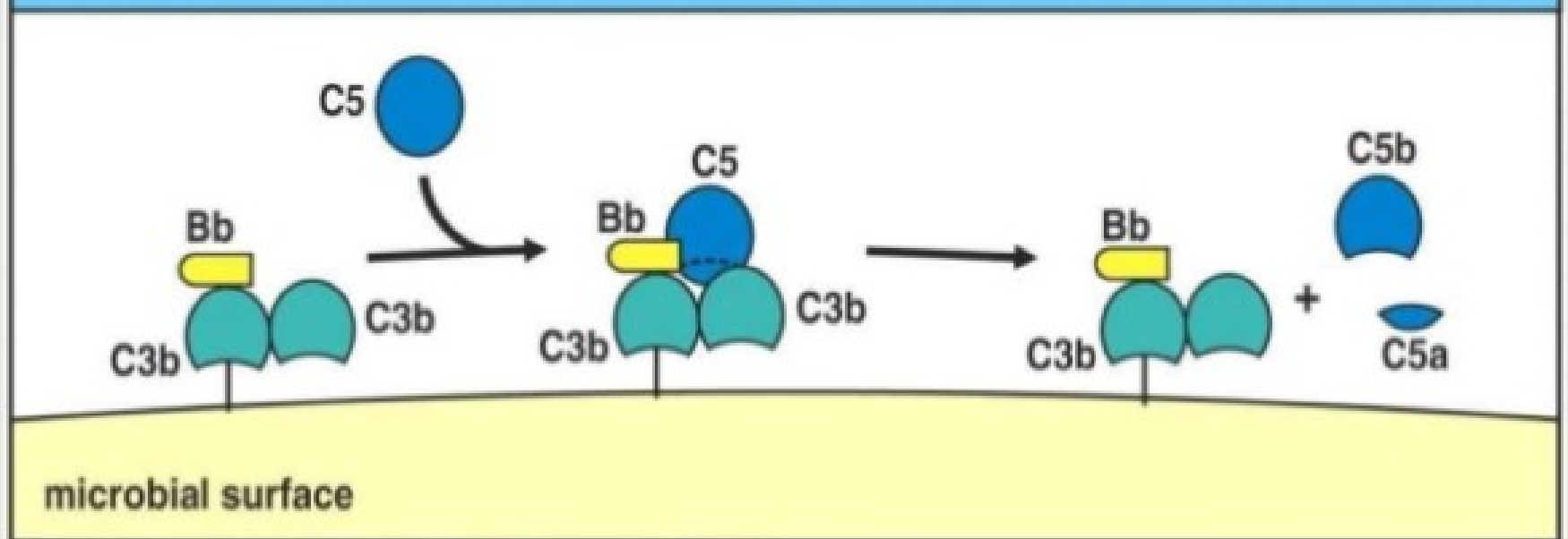
Properdin stabilizes C3 convertase C3bBb on a microbial surface



- ❑ Stabilizes C3b-Bb so it may cleave more C3.
May cleave over a million C3 molecules!
- ❑ Protects Bb-C3b from inactivation by complement control mechanisms.

C5 activation

C5 activation by the alternative C5 convertase



- C3b-C3b-Bb functions as the C5 convertase in the alternative pathway.

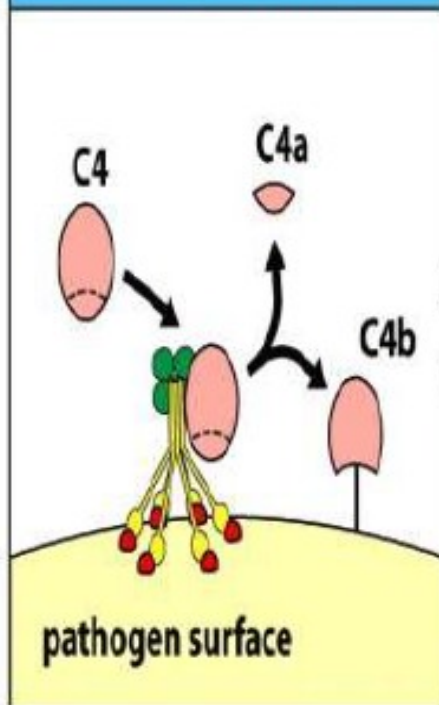
Lectin pathway



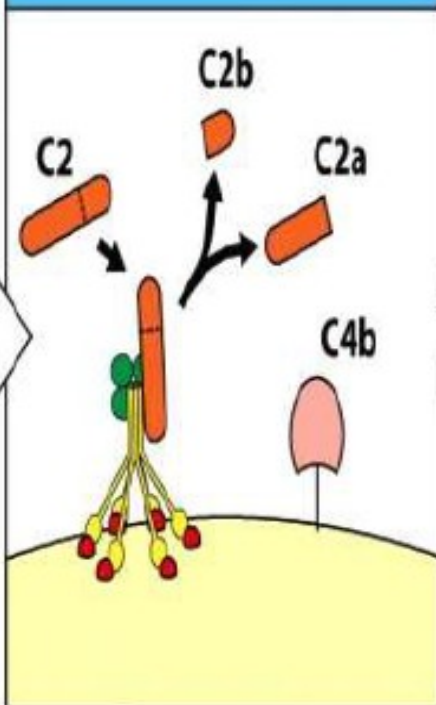
- Also known as the MBL Pathway
- In the lectin pathway, also known as mannose-binding protein) binds to the surface of microbes bearing mannan (a polymer of the sugar, mannose).
- Binding causes activation of MASP (MBP- associated serine proteases) cleave C2 and C4 and activate the classic pathway.
- Note that this process bypasses the antibody-requiring step and so is protective early in infection before antibody is formed.

Lectin Pathway

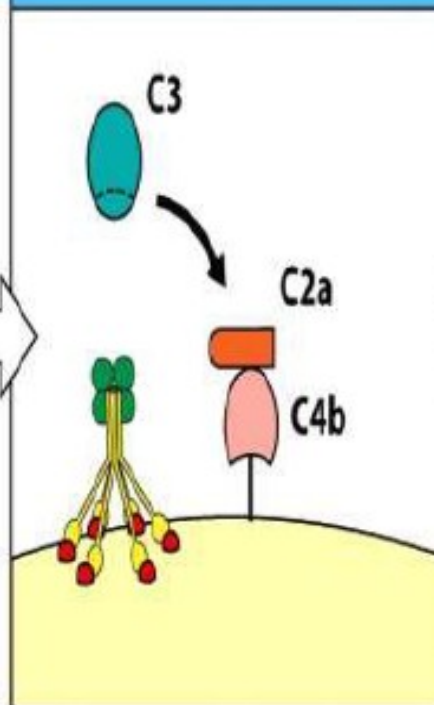
Activated MASP-2 cleaves C4 to C4a and C4b. Some C4b binds covalently to the microbial surface



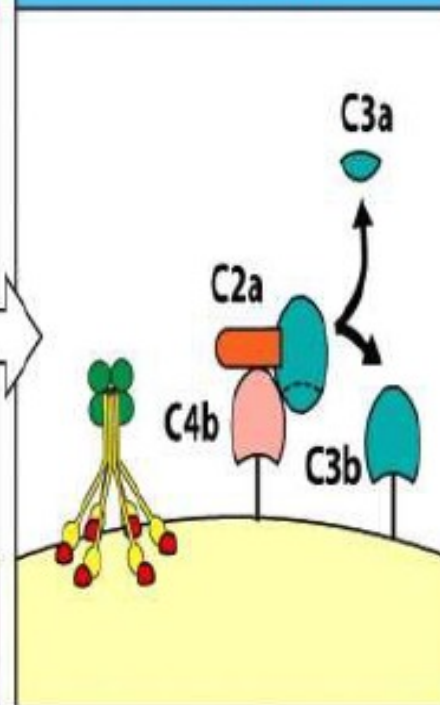
Activated MASP-2 also cleaves C2 to C2a and C2b



C2a binds to surface C4b forming the classical C3 convertase, C4b2a

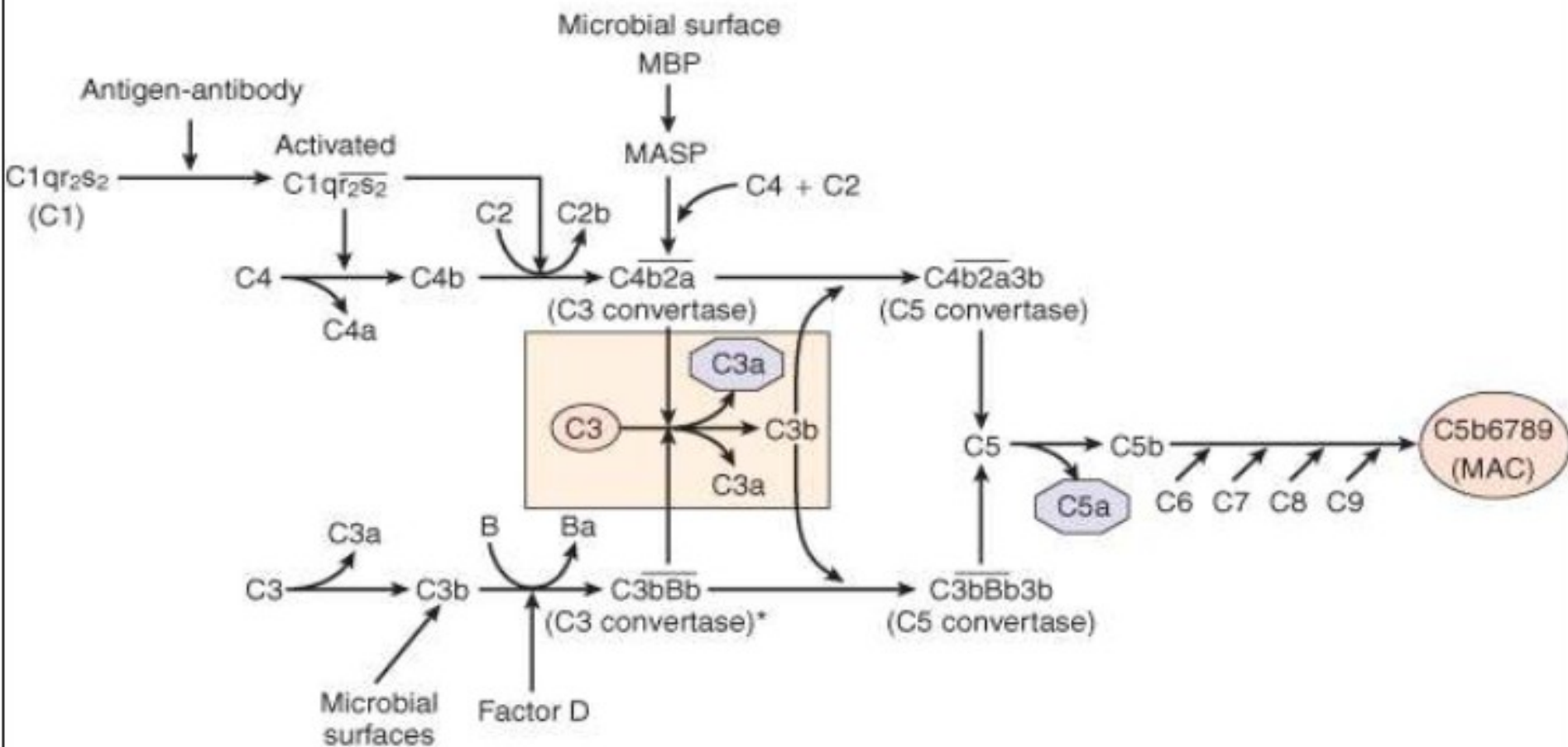


C4b2a binds C3 and cleaves it to C3a and C3b. C3b binds covalently to the microbial surface



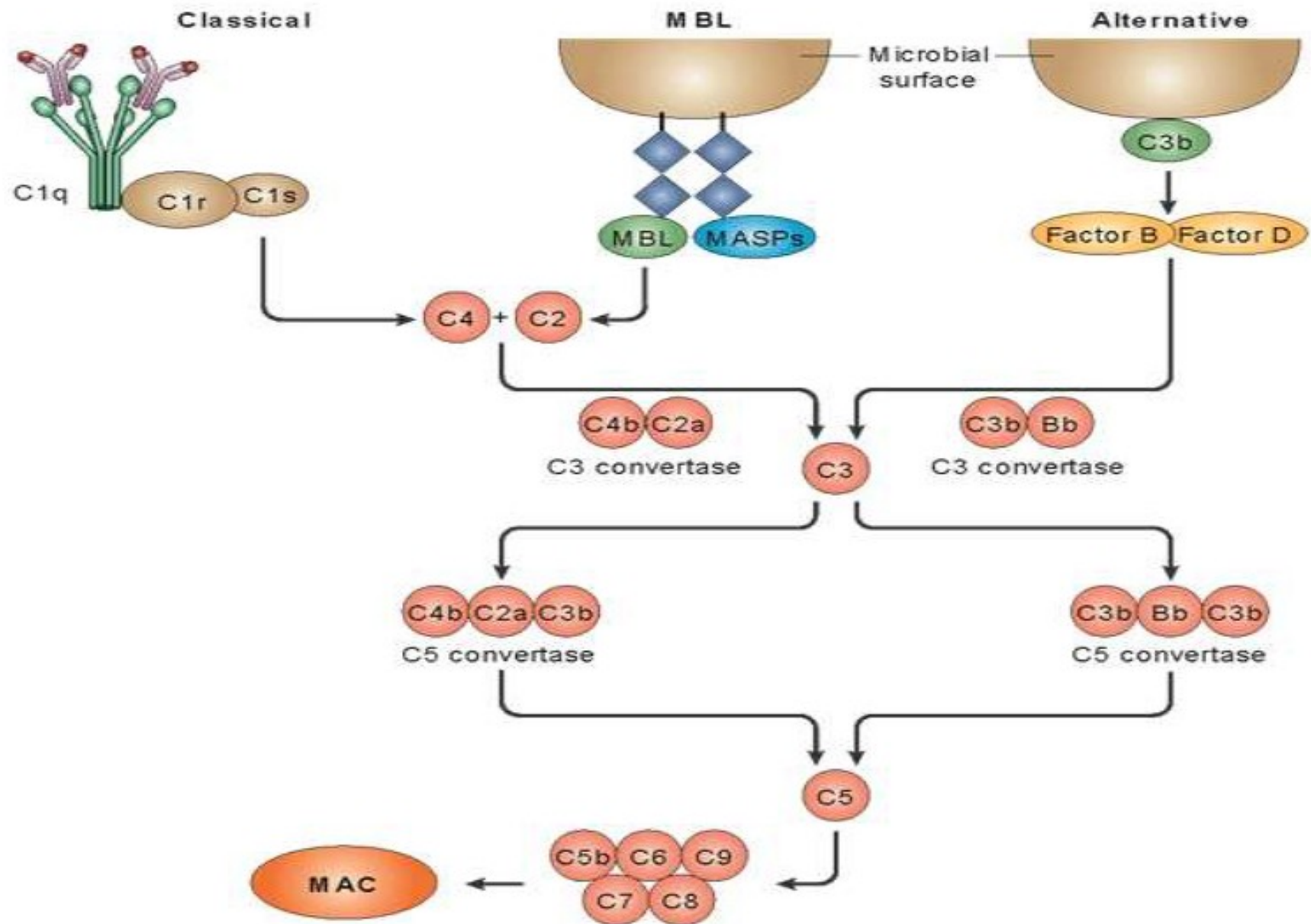
Classical Pathway

Lectin Pathway



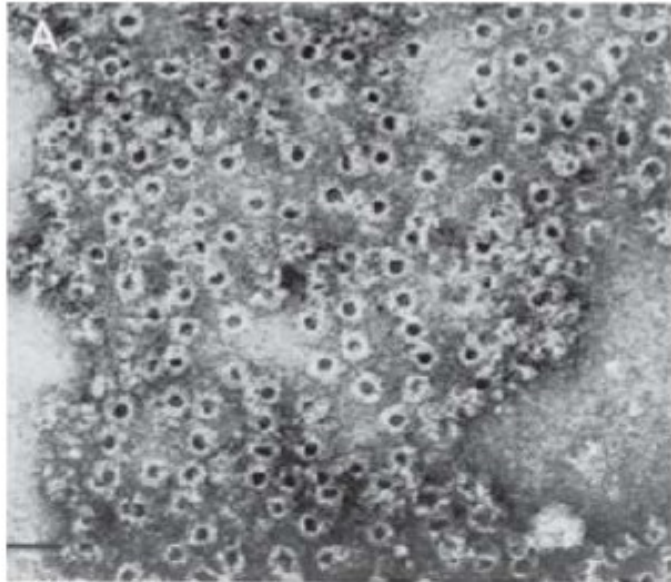
Alternate Pathway

* Stabilized by properdin

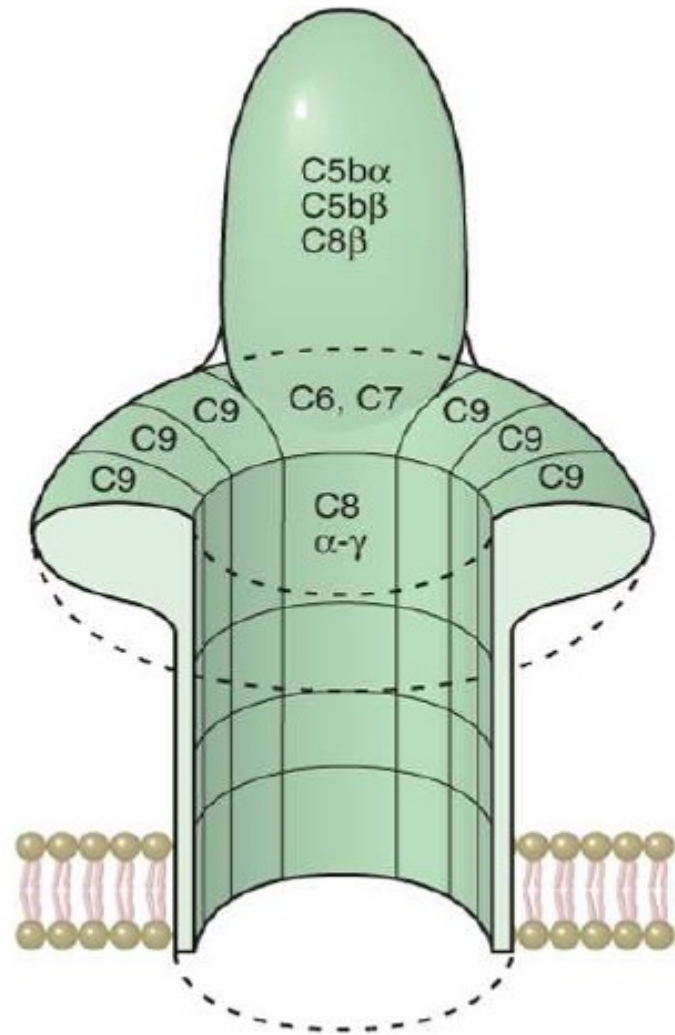




- Cleavage of C5 into C5a and C5b.
- C5 (structurally homologous to C3 and C4, lacks internal thioester bond)
- C5b initiates formation of MAC (complex of C5b, C6, C7, C8 and multiple C9 molecules) binds to C6, and C7 , recruits C8 and complex penetrates more deeply into the membrane.
- C9, a pore-forming molecule with homology to perforin.
- The complex of C5b678 forms a nidus for C9 binding and polymerization
- Penetrates membrane bilayers to form pores
- Disrupt the osmotic barrier, leading to swelling and lysis of susceptible cells



C





Biologic Effects of complement:

1. Opsonization

C3b & C1q; enhance phagocytosis

2. Chemotaxis

C5a and C5,6,7 complex → attract neutrophils

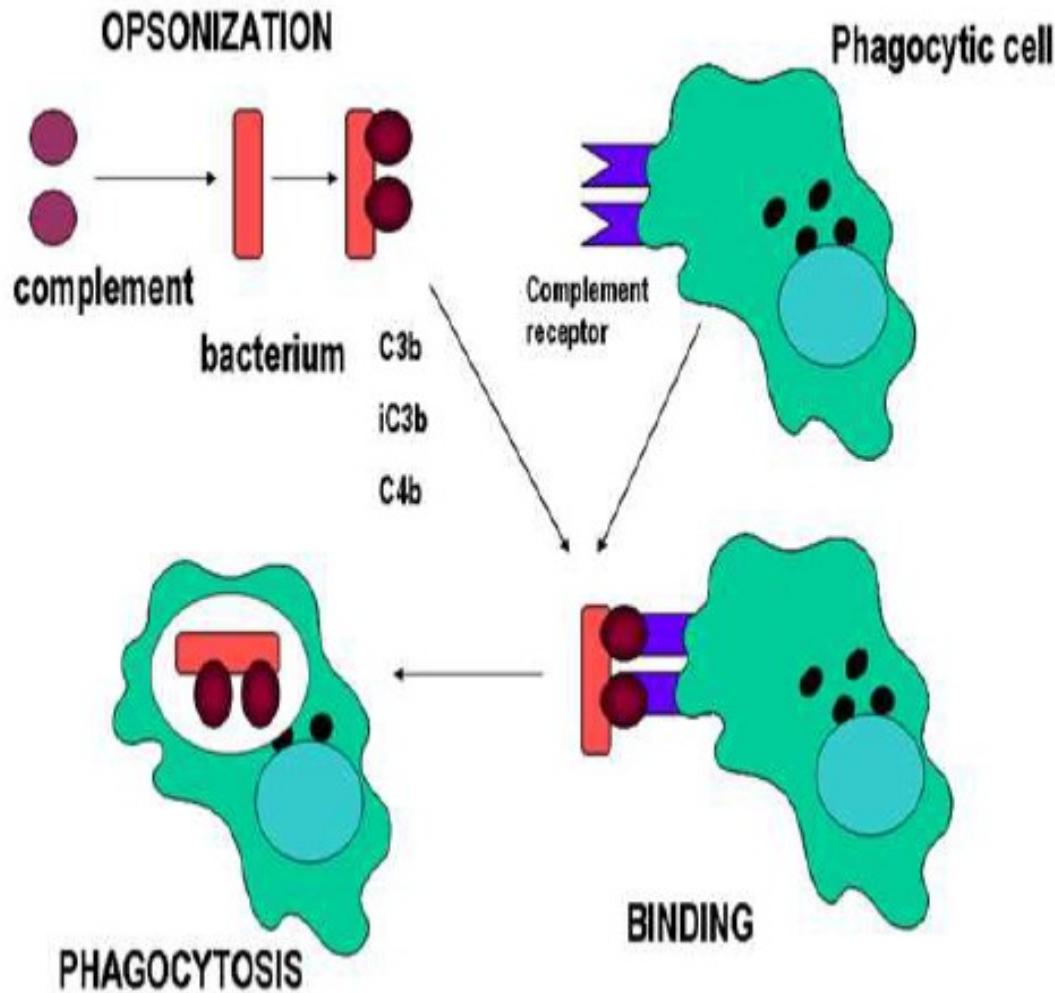
C5a – enhance adhesiveness of neutrophils to the endothelium

3. Anaphylatoxin (C3a, C4a, C5a)

Cause degranulation of mast cells

Bind directly to smooth muscles of bronchioles →
bronchospasm

Opsonization and phagocytosis



C3b is an opsonin

Opsonins are molecules that bind both to bacteria and phagocytes

Opsonization increases phagocytosis by 1,000 fold.



4. Cytolysis (MAC)

- Disrupt the membrane & the entry of water and electrolytes into the cell

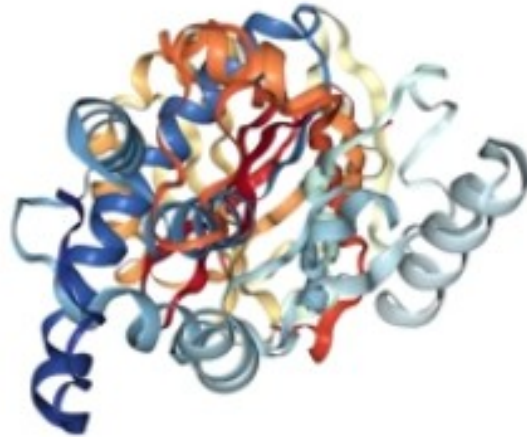
5. Enhancement of antibody production

- Binding of C3b to its receptors on the surface of activated B cells → enhanced antibody production

Differences Among the Complement Pathways

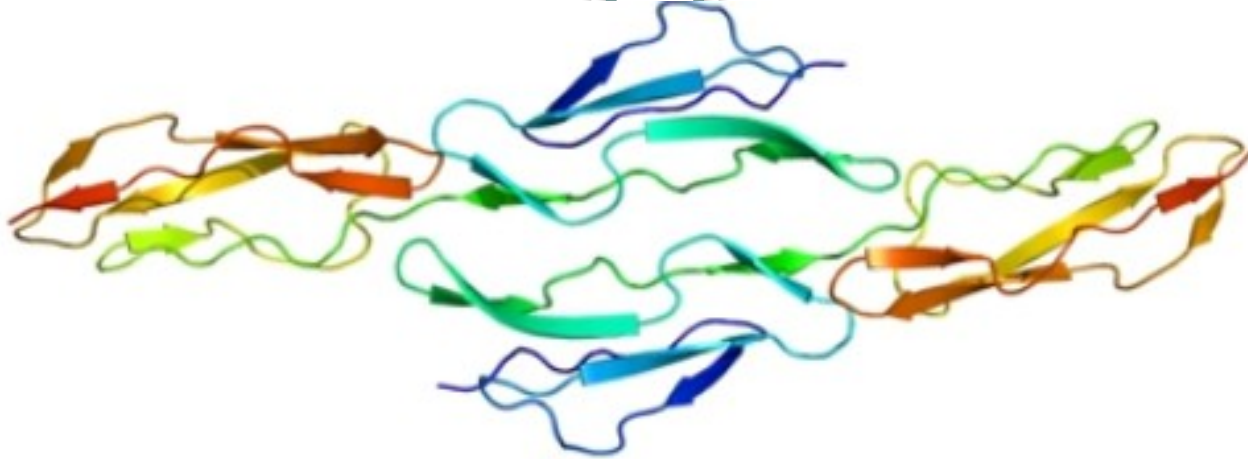
Differences	Classical Pathway	Alternative Pathway	Lectin Pathway
Enzymes	Uses C1-9 enzymes	Uses "Factor B", "Factor D" and "Factor P"	Uses MBL and MASP-1 and MASP-2
Surface Attachment	Ig binds to pathogen surface and facilitates binding of complement	Pathogen's cell surface creates microenvironment for complement activation	MBL binds to mannose containing polysaccharides on the pathogen's surface
Activation	• C1q binds Fc region of Ig • C1q activates C1r • C1r activates C1s • C1s cleaves C4 and C2 to form C3 convertase	• IgA/PG/LPS cleaves C3 • C3b binds Factor B • Factor B activated by Factor D • Resultant C3bBb is produced (i.e. C3 convertase)	• MBL binds mannose on cell surface • MBL activates MASP-1 • MASP-1 activates MASP-2 • MASP-2 cleaves C4 and C2 to form C3 convertase

Regulation of complement pathway



C1 inhibitor (serine protease inhibitor)

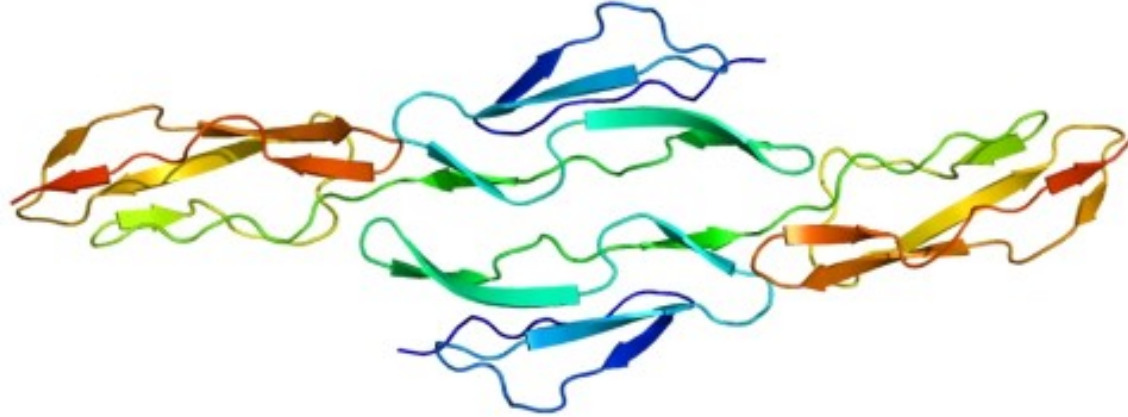
- binds active C1r:C1s
- dissociates it from Cq
- prevents it from cleaving C2 and C4



decay-accelerating factor

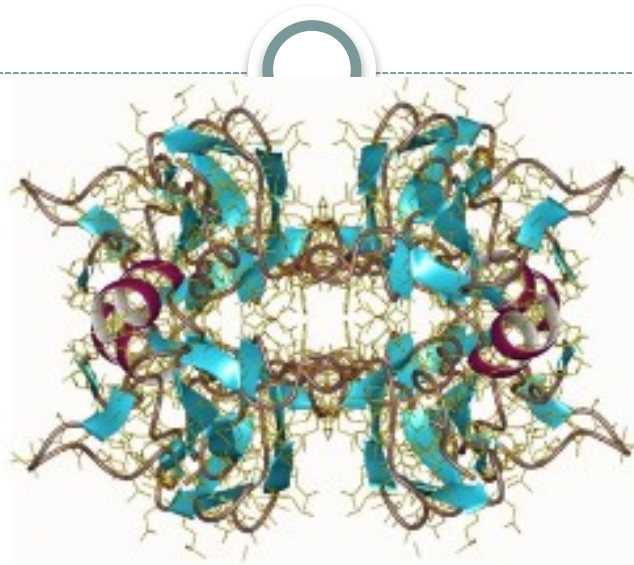
- competes with Factor B for C3b binding
- prevents alternative complement activation

displaces bound Bb to inactivate alternative C3 convertase



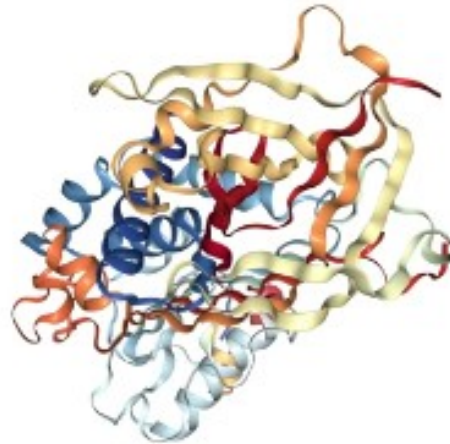
**decay-accelerating factor
C4-binding protein
complement receptor 1**

- these bind to C4b in classical C3 convertase and **displace C2a**



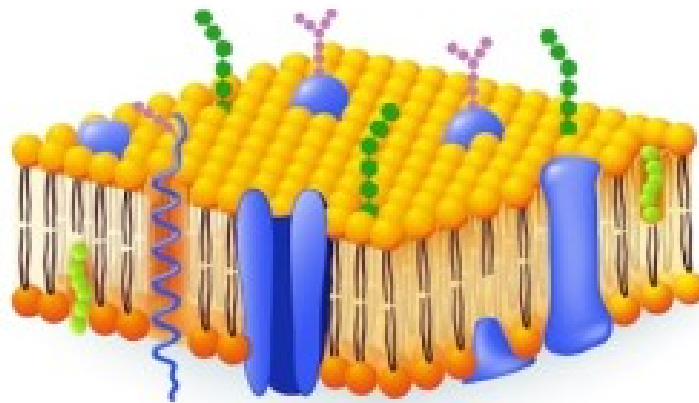
Factor H (plasma protein)

- outcompetes Factor B
- binds to C3b on host cells to prevent activation on them
- able to displace C3b from the C5 convertase



vitronectin (plasma protein)

- can bind to any C5b67 complex
- prevents them from inserting into host cell membranes
- **thereby inhibits the membrane attack complex**



protectin (membrane-bound protein)

- prevents C9 from binding to C5b678 complex
- **prevents complement activation from harming host cells**



Clinical Aspects of complement

1. Deficiency of C5-C8 & Mannan-binding lectin

- Predispose to severe *Neisseria* bacteremia

2. Deficiency of C3

- Severe, recurrent pyogenic sinus & resp. tract infections

3. Deficiency of C1 esterase inhibitor

- Angioedema → inc. capillary permeability and edema

4. Deficiency of DAF

- Increased complement-mediated hemolysis → paroxysmal nocturnal hemoglobinuria



5. Transfusion mismatches

- Activation of complement → generate large amounts of anaphylatoxins & MAC → red cell hemolysis

6. Autoimmune diseases

- Immune complexes bind complement → low complement levels + activate inflammation → tissue damage

7. Severe liver disease

- Deficient complement proteins → predispose to infection with pyogenic bacteria



Thank you

Tolerance and Autoimmunity

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MSc Integrated Immunology, University
Of Oxford.

Learning outcomes

- Understand the concept of tolerance and how lymphocytes distinguish self from non self.
- Understand mechanisms of central and peripheral tolerance.
- Understand proposed mechanisms of autoimmune disease development.
- Examples of common autoimmune diseases.

Tolerance

- **Self-tolerance** is the state of specific unresponsiveness to self-antigen.
- The immune system developed to protect against foreign organisms and spares the individuals' own components a.k.a self antigens/autoantigens.
- Both the innate and adaptive immune responses have evolved to make this distinction.

Tolerance

- Self-tolerance is a property of both B and T lymphocytes.
- It occurs when the interaction of an autoantigen with self-antigen specific lymphocytes (BCR/TCR) fails to activate the lymphocytes thus they become **tolerized**.
- Stages of tolerance: **central** and **peripheral**

How do lymphocytes distinguish self from nonself?

- Self-antigens have no specific characteristics that distinguish them from foreign antigens.
- lymphocytes rely on other characteristics to make this distinction -possible scenarios are:
 1. Mature vs. immature lymphocytes: exposure of *immature* lymphocytes to antigens results in tolerance, whereas the first exposure of *mature* lymphocytes to the same antigen results in activation.

Cont.

2. **Continuous vs. short antigen exposure:**
Lymphocytes are continuously exposed to high levels of self-antigens, while exposure to foreign antigens is generally abrupt and short lived.
3. **Costimulation:** If the antigen is encountered in the absence of co-stimulatory signals, then the lymphocytes are tolerized; if antigen is encountered in the presence of co-stimulatory signals, the lymphocytes are activated.

Central Tolerance

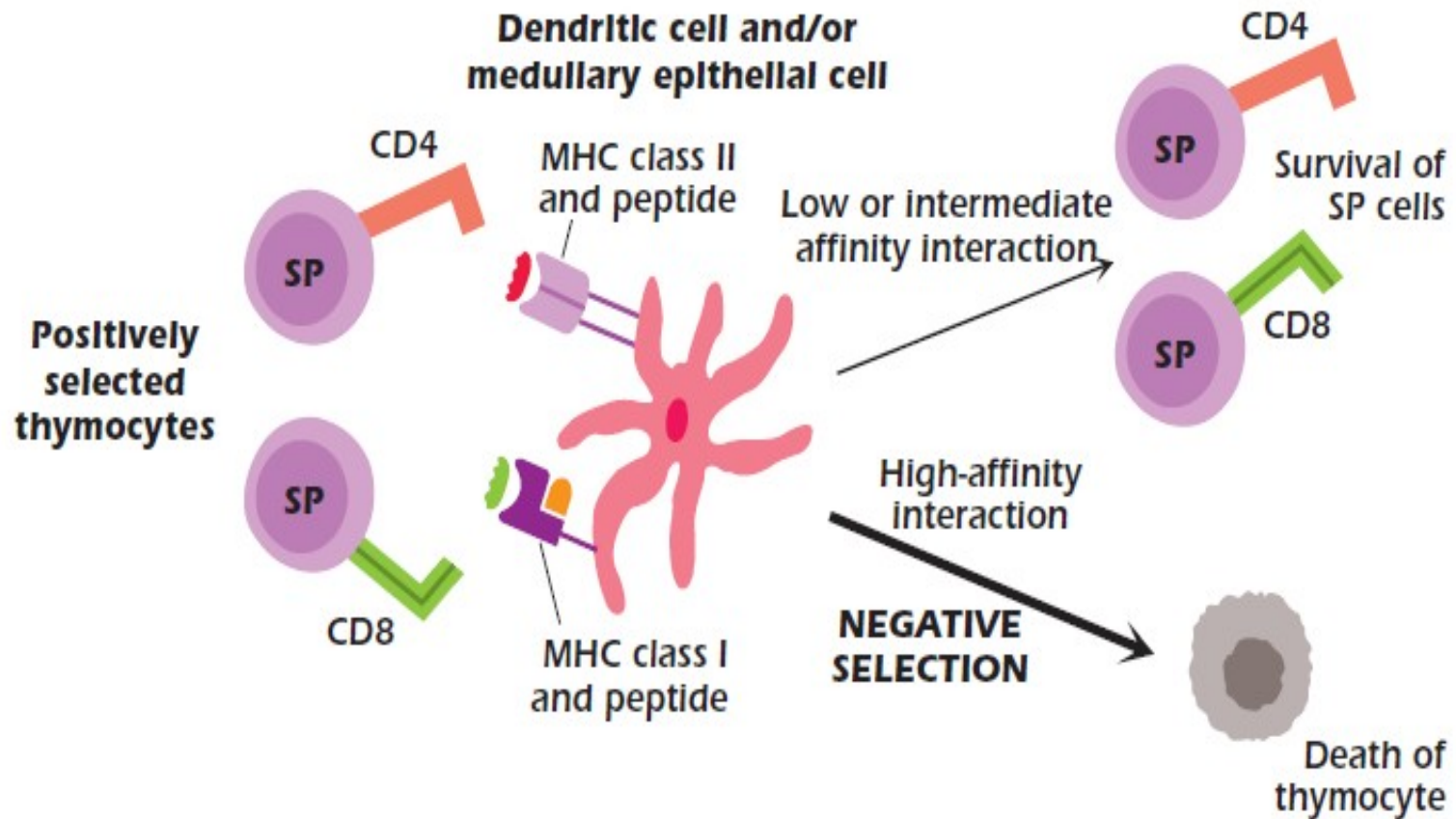
- Central tolerance is induced during the early stages of development.
- Occurs in the primary lymphoid organs (BM for B cells and the thymus for T cells) as part of negative selection process.
- Negative selection ensures that the majority of B and T cells that leave to the periphery react only with foreign antigens.
- The strength, or **avidity**, of interaction of the BCR or TCR with its autoantigen influences cell fate.

Mechanisms of Central Tolerance

First: Deletion.

- Deletion is a mechanism by which autoreactive T and B cells are eliminated from the repertoire by undergoing apoptosis.
- For T cells it occurs in the thymus: it is the major mechanism of negative selection for developing thymocytes, facilitated by **AIRE** gene.
- For B cells it occurs in the BM- CT in B cells shows some features that are not found in the development of CT in T cells e.g. anergy and receptor editing.

Mechanisms of Central Tolerance: T cells



Mechanisms of Central Tolerance

Second: Anergy

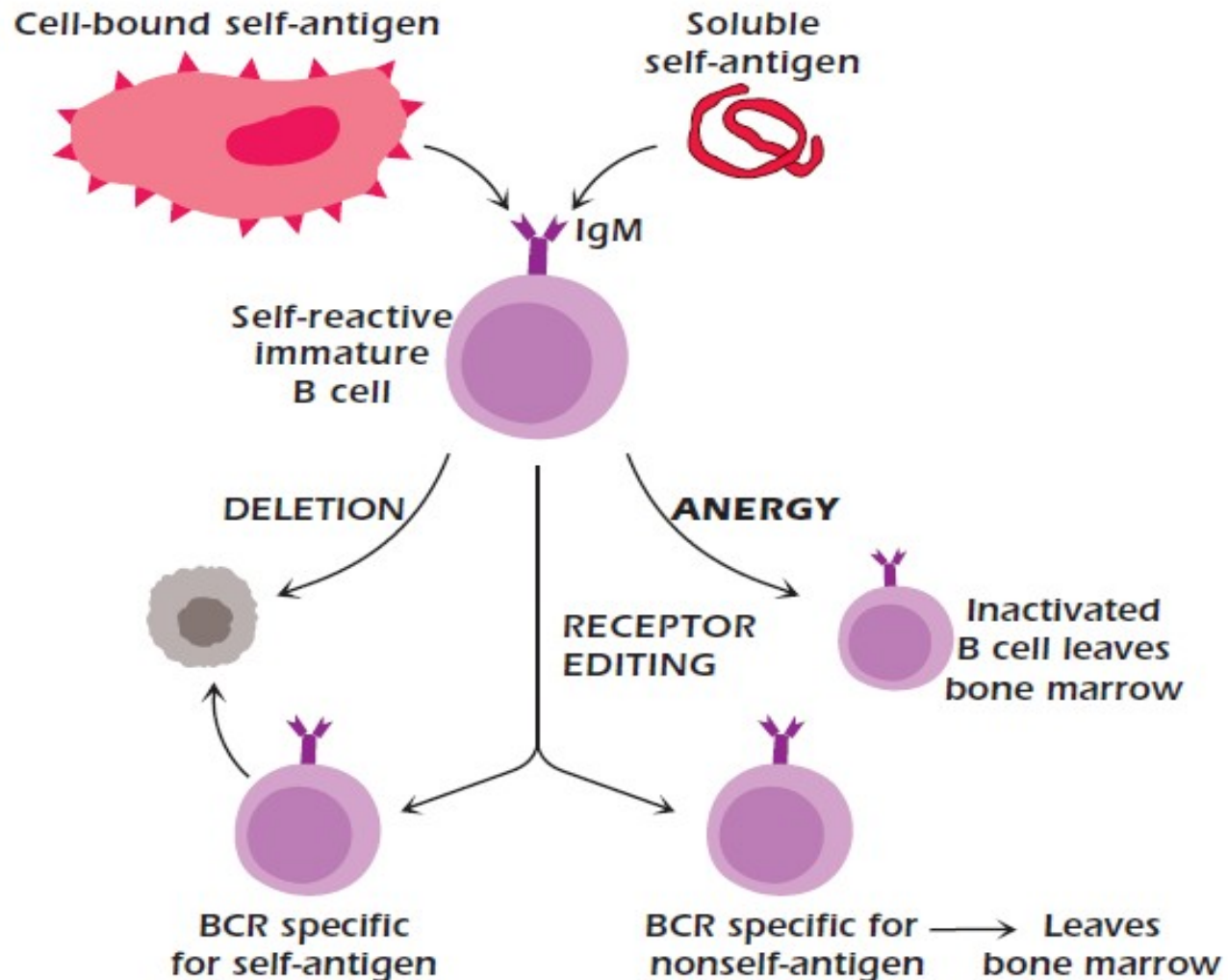
- The functional inactivation of a lymphocyte, resulting in unresponsiveness upon further contact with antigen- depends on the extent of BCR cross-linking by the antigen.
- Anergic B cells cannot be activated to proliferate and differentiate into antibody-secreting cells when BCR is engaged.
- Anergy does not seem to be a mechanism of central tolerance for developing T cells.

Mechanisms of Central Tolerance

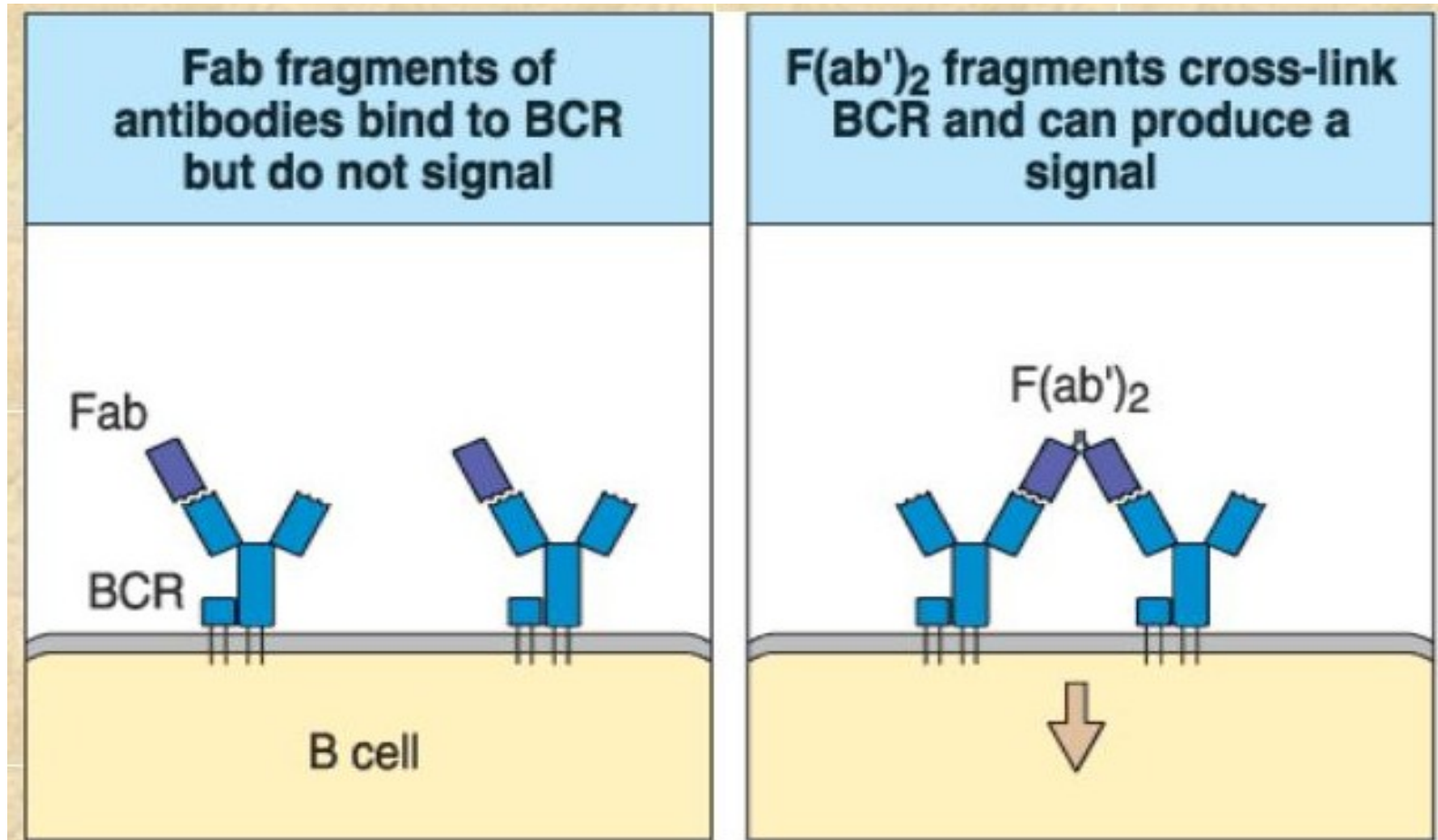
Third: Receptor editing.

- It allows an immature B cell expressing a BCR specific for a self-antigen to replace it with a BCR specific for a nonself-antigen.
- Occurs predominantly in the variable region of the light chain genes.
- Developing T cells do not appear to use receptor editing.

Mechanisms of Central Tolerance: B cells



Receptor cross-linking



Cross-linking - bringing together more than one BCR complex in the cell membrane.

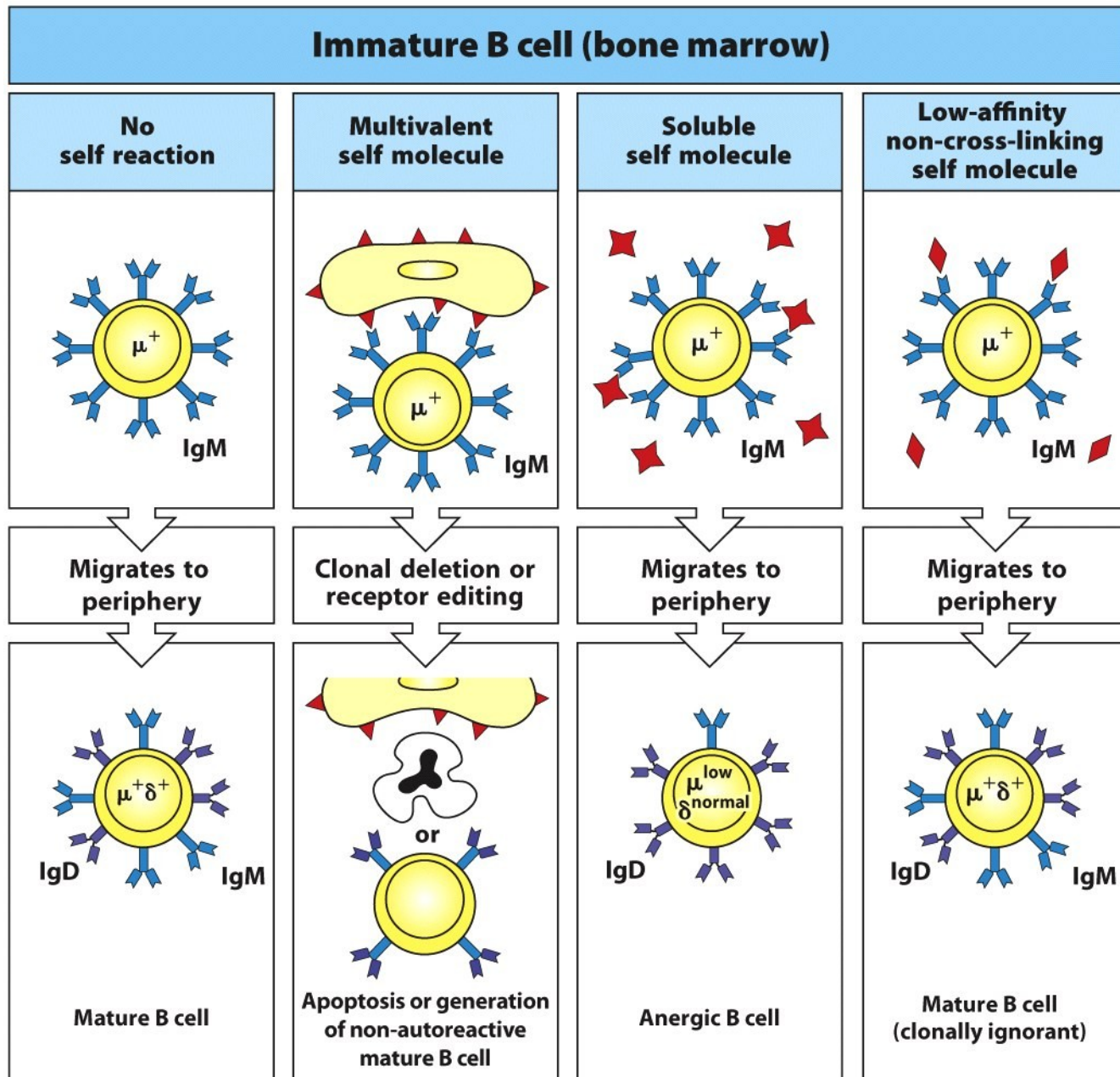


Figure 8.12 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Peripheral tolerance

- Tolerance that is induced in mature lymphocytes.
- Central tolerance processes are not perfect: some autoreactive B cells and T cells escape negative selection and enter the periphery.
- How?? Low level of the self antigen in PLO or weak affinity of developing lymphocytes.
- Peripheral tolerance mechanisms keep them under control.

Peripheral tolerance

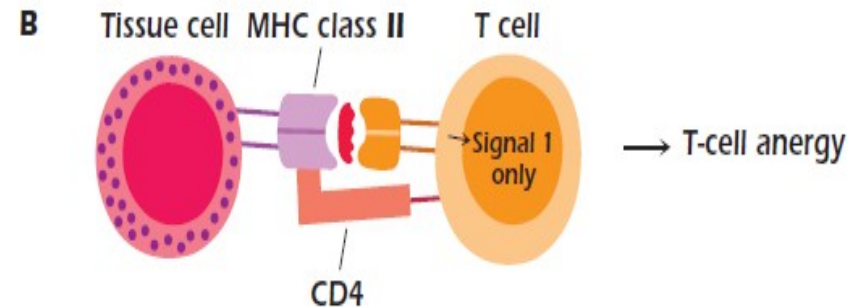
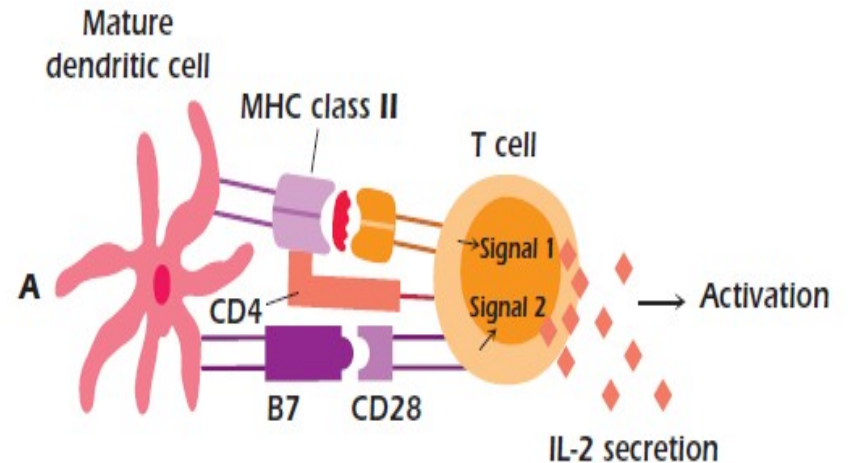
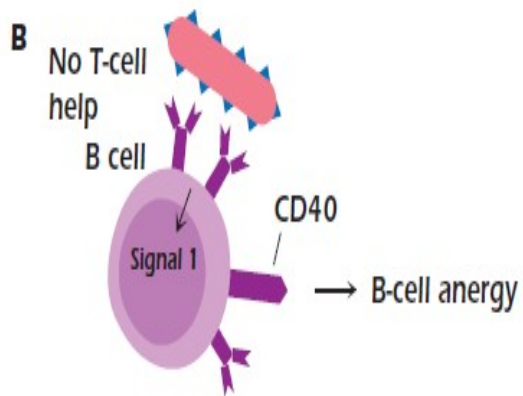
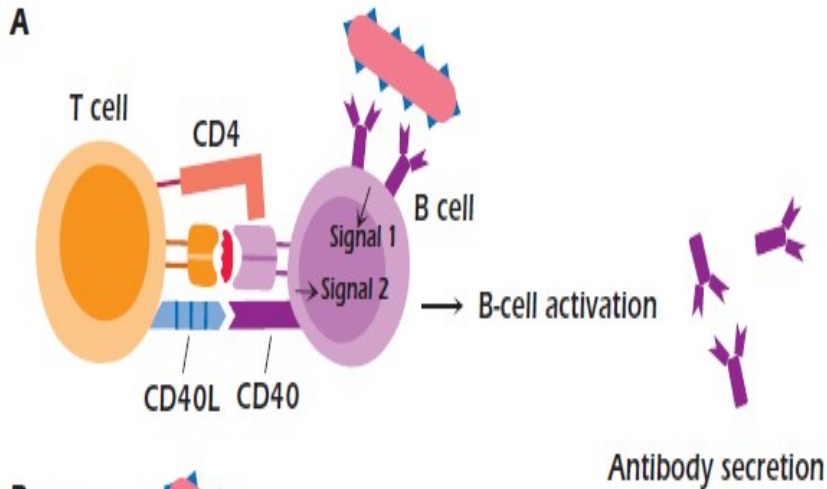
- Peripheral tolerance work as a safety net to catch autoreactive B and T cells that escape to- or arise in the periphery.
- It also prevents responding to every foreign antigen encountered, such as those in our food.
- Mechanisms of peripheral tolerance include: anergy, Fas-mediated activation of apoptosis, and the induction of regulatory T (iTreg) cells.

Mechanisms of peripheral tolerance

First: Anergy

- T-cell anergy is a major mechanism of tolerance to self-antigens seen in the periphery.
- The two signals theory: if a tissue cell or unactivated APC presents antigen to a T cell, the T cell will be anergized (no co-stimulatory signal delivered).
- Likewise, B cells specific for TD antigens require two signals to be activated: the first signal provided by antigen, and the second signal by T cells (mainly CD40-CD40L interaction).
- B cells exposed to self-antigen become anergized in the absence of T-cell help (the autoreactive T cells are usually not available as they are deleted in the thymus) .

Mechanisms of peripheral tolerance



Mechanisms of peripheral tolerance

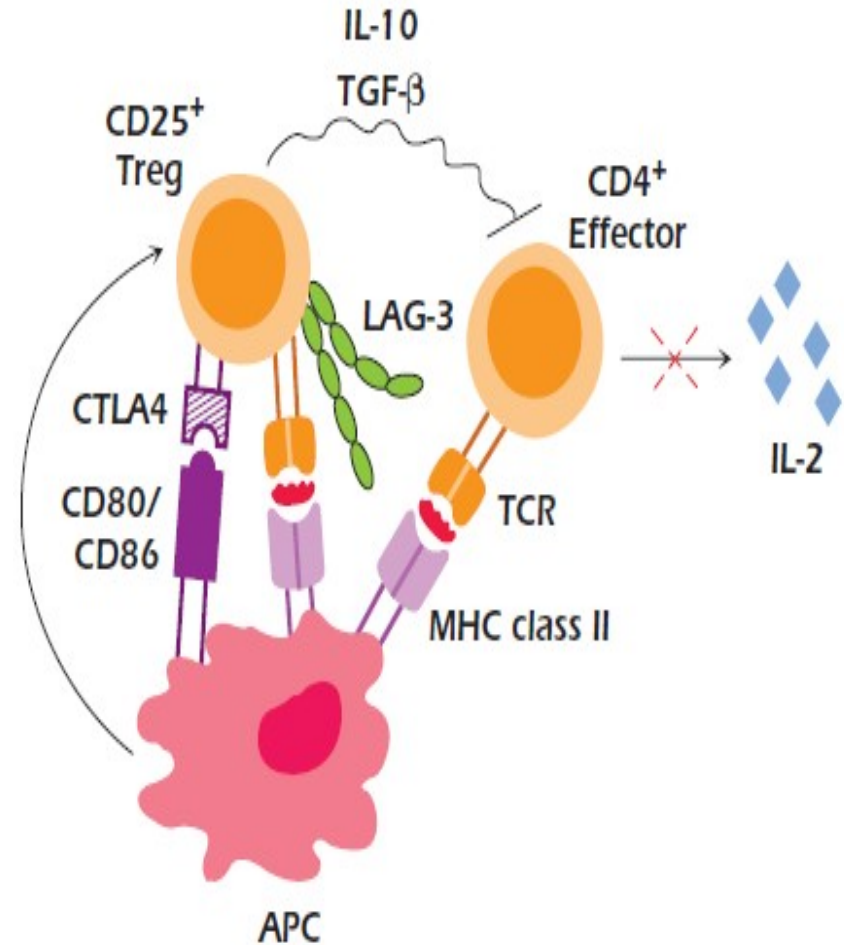
Second: regulatory T cells.

- Referred to as T suppressor cell.
- Once activated, their suppressive function is antigen non-specific: can suppress autoreactive CD4+ & CD8+ T cells that are specific for a different antigen.
- Can also suppress B cells, DCs, and NK cells.

Mechanisms of peripheral tolerance

- **Proposed mechanisms of the suppressive function of Treg cells:**

1. They receive a signal via CTLA-4 that induces their suppressive activity.
2. They receive a signal triggering their suppressive activity when LAG-3 interact with an MHC II.
3. The secretion of the inhibitory cytokines IL-10 and TGF- β suppress CD4⁺ T cells activation.



Mechanisms of peripheral tolerance

Third: Fas–FasL Interactions.

- **Fas (CD95)** is expressed by activated lymphocytes (T-&B- cells).
- Fas binds to **Fas ligand** (FasL, CD178), expressed by several cell types e.g. activated T cells and some epithelial cells.
- The ligation of Fas initiates apoptosis in the cell expressing Fas.
- Mutation in Fas gene result in autoimmunity: autoreactive B and T lymphocytes are not properly deleted in the periphery.

Oral Tolerance

- Oral tolerance is specific immune unresponsiveness to ingested food antigens.
- It is mediated by T cells and other immune cells of the GALT.
- It is dependent on the dose of ingested antigen.
- A low dose of ingested antigen induce Treg cells, while a high dose induce T-cell anergy or deletion.

Oral Tolerance

- The cytokine milieu of the GALT contains elevated levels of IL-4, IL-10, and TGF- β : this promotes differentiation into TH2 and Treg cells and inhibits TH1 cells.
- The Treg cells induced are specific for the ingested antigen: however, they mediate nonspecific suppression by secreting TGF- β .

Autoimmunity

- Autoimmunity refers to immune responses to self.
- Autoimmunity does not necessarily lead to disease.
- For example, low levels of autoantibodies are present in most individuals - significant levels of antinuclear antibodies or rheumatoid factor (anti-self IgG) are found in healthy individuals.
- Similarly, T-cell reactivity to self antigens may occur in the absence of overt disease.

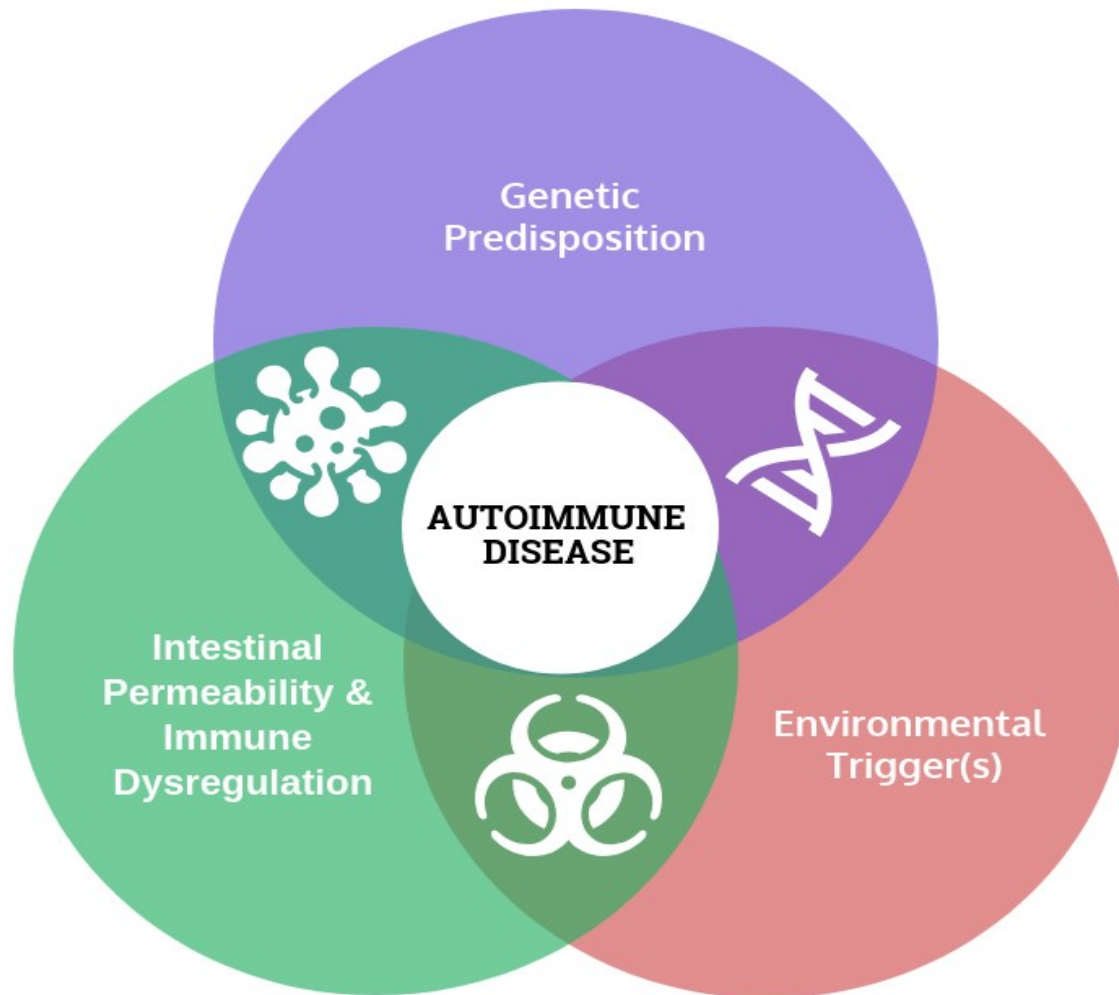
Autoimmunity

- The suggestion that autoimmunity is the cause of the disease came from experiments/observations when transfer of autoantibodies or autoreactive T cells from patients with autoimmune disease to a healthy individual reproduced the disease manifestation in the healthy recipient.
- E.g. neonatal graves disease, neonatal myasthenia gravis- induced by trans-placental IgG transfer from the affected mother.

Autoimmunity and disease

- The etiology of autoimmune diseases is complex and not completely unraveled.
- Most AID are caused by a combination of genetic and environmental factors.
- AID are rarely caused by an anomaly of a single gene- certain gene defects may predispose an individual to autoimmunity but exposure to an environmental factor is necessary to precipitate the disease.

What causes autoimmune disease



AID: Genetic Susceptibility

- Genetic predisposition to AID is indicated by higher prevalence of AID in monozygotic twins and its clustering in families.
- The inheritance pattern is complex: meaning that AID involve the products of multiple genes and no individual gene is sufficient to elicit the disease.
- AID are genetically heterogeneous: the same clinical disease may result from the combined effect of different genes.

Genetic Susceptibility: HLA genes

- The most studied genes for association with AID are the HLA genes (the human MHC).
- The importance of HLA molecules in peptide presentation to the TCR and shaping the TCR repertoire explains their role in AID development.
- Some HLA alleles are better than others at presenting self-peptides to autoreactive T cells- thus predisposing to autoimmunity.

Genetic Susceptibility: HLA genes

- Genetic susceptibility to specific AID is usually linked to HLA class II alleles- some HLA I alleles are also involved e.g HLA-B27.
- Linking AID to a specific HLA allele, does not mean that every individual expressing the allele develops disease.
- Other genes or certain environmental triggers are also required.

Environmental Susceptibility

- Some environmental factors can trigger autoimmunity- infectious and non infectious triggers.
- **Suggested mechanism of how environmental factors trigger AID:**
 1. Inducing the release of sequestered antigens
 2. Molecular mimicry
 3. polyclonal activation.

Environmental Susceptibility

First: Release of sequestered antigens

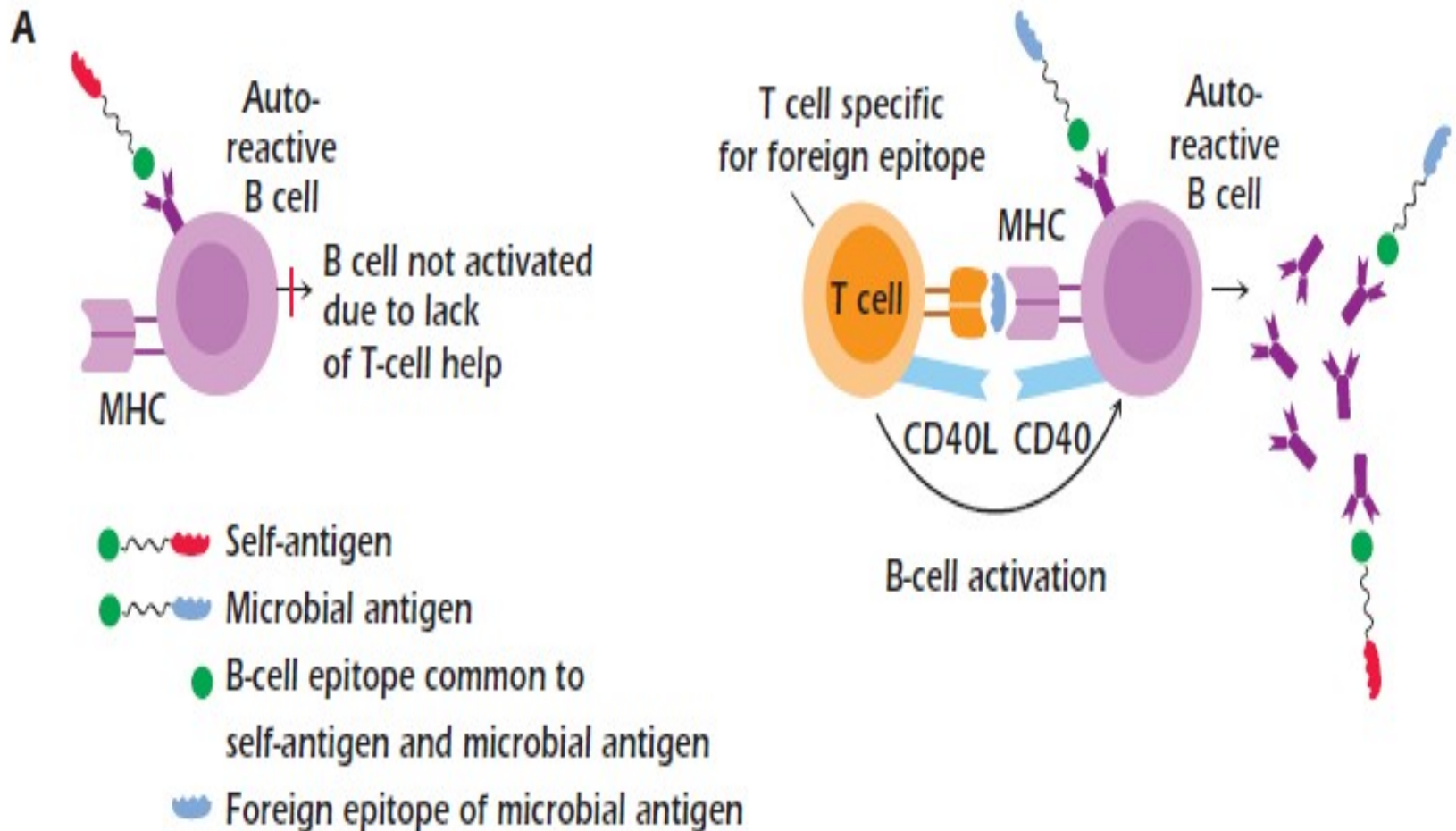
- Some autoantigens are protected from the immune system because they are found in privileged sites- e.g. antigens in eye, gonads, chondrocytes in the cartilage, cardiac and neuronal antigens.
- Thus, even if there is autoreactive T and B cells specific for these antigens, they will not be activated to initiate autoimmunity- No access for the autoantigen.
- These antigens may become exposed by physical injury or infection —an autoimmune response may result.
- For example: cases of autoimmune pericarditis and myocarditis seen following myocardial infarction or cardiac surgery.

Environmental Susceptibility

Second: molecular mimicry.

- T cells that recognize a foreign (usually microbial) antigen containing an epitope that cross-reacts with self-antigen- they can subsequently activate autoreactive B cell to produce autoantibodies.
- The epitope on the microbial antigen is similar to the epitope on the self-antigen - the antibodies made may interact with both self-antigens and foreign antigens.
- E.g. Rheumatic fever sometimes develops after a streptococcal infection: antibodies to the M protein of *Streptococcus pyogenes* cross-react with cardiac myosin causing heart damage.

Environmental Susceptibility



Environmental Susceptibility

Other mechanisms: Bystander activation

- Activation of T cells specific for an antigen *X* during an immune response against antigen *Y*.
- May occur during viral infections.
- Exact mechanism is unknown

Environmental Susceptibility

- Noninfectious triggers of autoimmunity include hormones and drugs.
- Most autoimmune diseases are much more common in women than in men- e.g. in SLE it is close to 10:1 and the disease may be exacerbated by estrogens.
- Certain drugs can chemically alter the epitope of a self antigen to render it immunogenic, resulting in autoimmunity- e.g. penicillin induced haemolytic anaemia and drug induced lupus.

Autoimmune diseases

- **The breakdown of immunological self-tolerance leads to a cascade of events:**
 - Presentation of host antigens.
 - Activation and expansion of autoreactive T cells.
 - Release of pro-inflammatory cytokines.
 - Differentiation of self-reactive B cells with production of organ-specific autoantibodies.
- These events are responsible for the classic tissue damage observed in autoimmune diseases, leading to dysfunction of the target organ.

Mechanisms of autoimmune tissue injury

- Tissue damage in autoimmune diseases can occur through several mechanisms, which are analogous to three of the classical types of hypersensitivity reactions:
- Type II: caused by antibodies against altered self proteins as in Graves disease, myasthenia gravis.
- Type III: caused by immune complexes (antigen-antibody complexes) as in SLE.
- Type IV: delayed-type hypersensitivity, mediated by T cells e.g. IDDM and Hashimoto disease, multiple sclerosis.

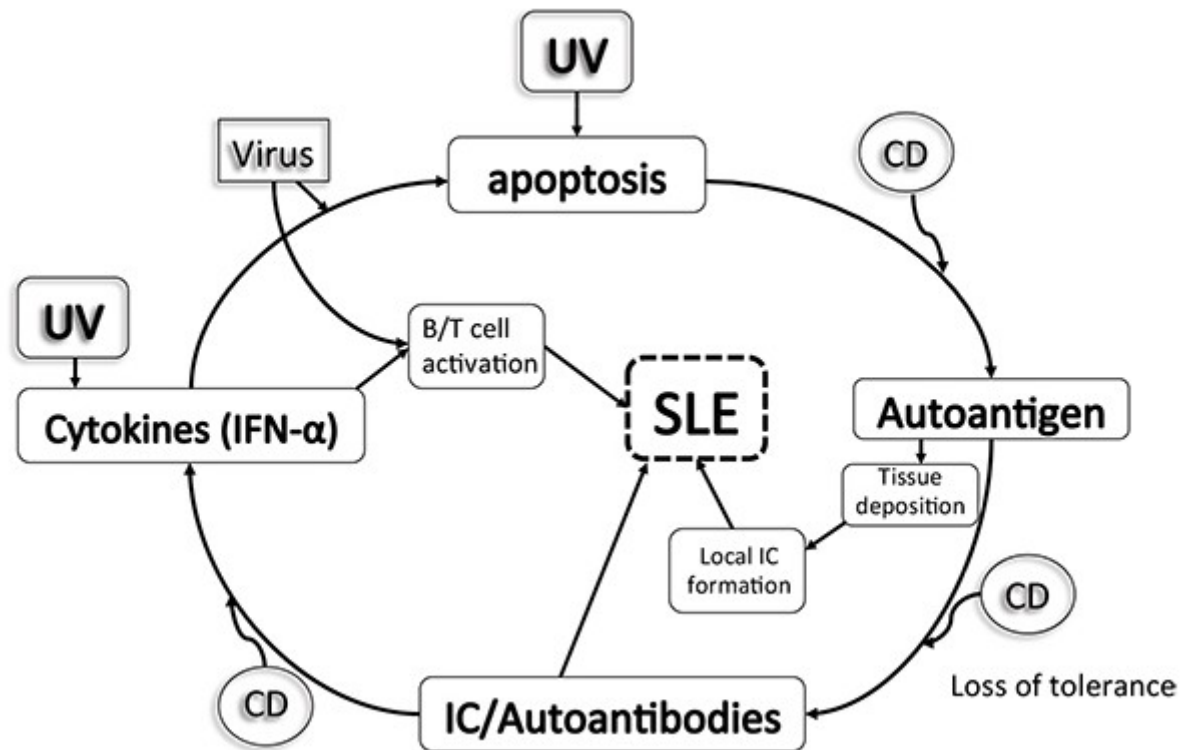
Examples of Autoimmune diseases

1. Systemic Lupus Erythematosus

- An autoimmune disease mediated mostly by autoantibodies and immune complexes (Ag-Ab).
- **Autoantigens:** components of the cell nucleus mainly DNA resulting in antinuclear antibodies ANA, anti-dsDNA antibodies.
- **Triggers:** infection-EBV, UV radiation, HLA-II alleles.
- **Target organ/s:** systemic, secondary to immune complex deposition in the skin, joints, lungs, blood vessels, heart, kidney, and brain.



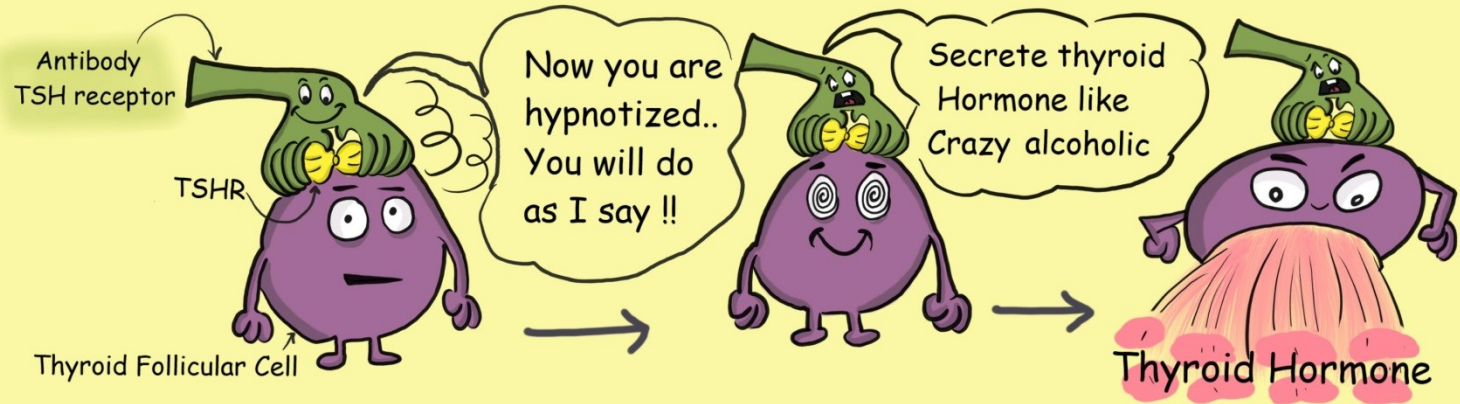
Systemic Lupus Erythematosus



Examples of Autoimmune diseases

2. Myasthenia Gravis

- An AID that is mediated by antibodies.
- **Autoantigen:** the acetylcholine receptor at neuromuscular junctions.
- **Autoantibodies:** blocks the binding of acetylcholine (ACh) to the receptor(antagonists)- thus prevent nerve impulse transmission causing muscle weakness.
- There is association with thymoma and HLA-DR3 alleles.



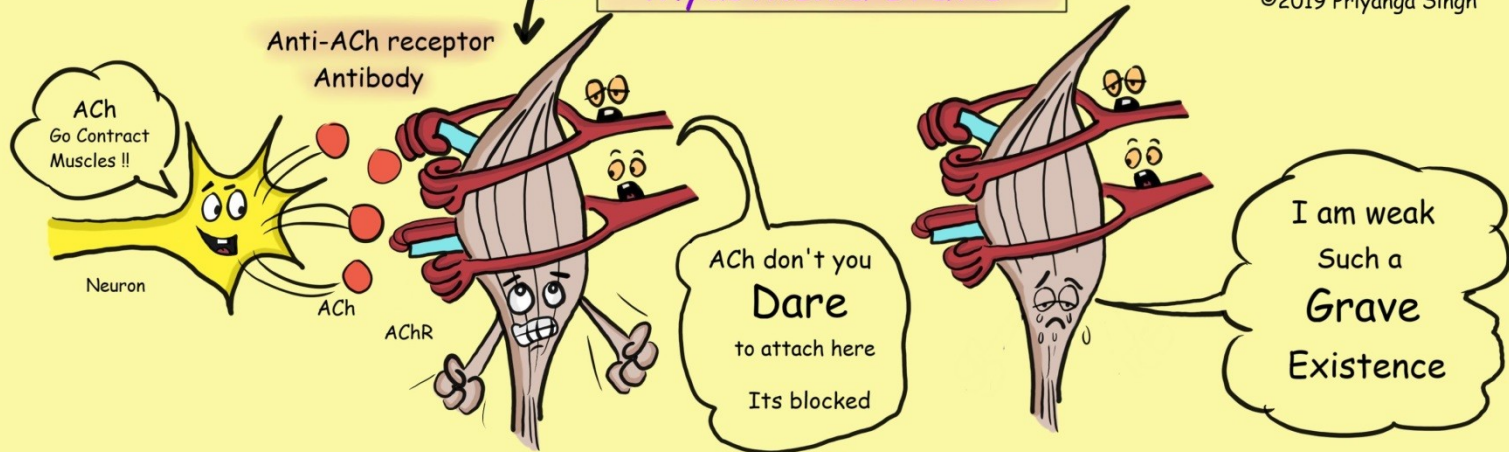
Antibody mediated Cellular Dysfunction

Type II Hypersensitivity

Graves Disease Hyperthyroidism

Myasthenia Gravis

Creative-Med-Doses
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Examples of Autoimmune diseases

3. Type I Diabetes Mellitus.

- An organ specific AID that involves T-cell mediated -CD8+ & CD4+ T cells- destruction of the pancreatic islets β cells.
- Autoantigen: β islets antigens e.g. GAD & insulin.
- Anti-insulin and anti-GAD autoantibodies are detected- they arise as a consequence of β -cell destruction and are not the initial cause of the destruction
- Triggers: HLADR3/DR4, infections-coxsackie virus

Examples of Autoimmune diseases

4. Rheumatoid Arthritis.

- An AID that causes chronic inflammation of the joints.
- The pathogenesis of RA is complex : it involves T-, B-lymphocytes and macrophages.
- Most patients produce IgM antibody specific for a determinant on the Fc portion of IgGs. This anti-IgG antibody is called **rheumatoid factor** (RF).
- When RF binds to IgG, the resulting immune complexes deposit in the joints, it recruit complement and establish an inflammatory process.

